



22 February 1979

Industrial innovation in the UK—a first step

OVER the past few years, Britain's faltering industry has come in for much attention from a variety of official and semi-official bodies. There has been the establishment of a National Enterprise Board and several discussion documents on the engineer and his relation with universities, industry and government, culminating in the Finniston Committee of Enquiry. The Science Research Council has taken a new range of initiatives such as the Co-operative Awards in Science and Engineering scheme, Total Technology courses and Teaching Companies. Now from the very centre of government, the Cabinet Office, comes a paper *Industrial Innovation* (HMSO, £1) produced by an unnamed working party, chaired by Dr A. Spinks (ICI), for the Advisory Council for Applied Research and Development (ACARD). The working party had a brief to consider ways in which conditions for innovation in British industry might be improved and to make appropriate recommendations for Ministerial action.

'Innovation', in current usage, means much more than just invention. It encompasses improvements to present products and processes as well as the introduction of new ones, and innovation does not just comprise what goes on in the R & D laboratory; it includes all the activity that goes into getting the new or improved commodity widely accepted. Some relatively unspectacular activities such as making car doors that shut with a satisfying click, or mechanical devices that break down less frequently than their competitors may be much more profitably innovative than no end of ultra-high technology. Identifying niches that no-one else has explored, be they relatively humble in technological terms, may bring major rewards. Maybe a slower but much cheaper propeller plane would be a better project for the 1980s than another supersonic jet transport.

It is not only Britain that is seriously worried about its industry's record. The United States is equally concerned about overseas competition from Japan and Western Europe, even with the built-in help of an enormous and affluent domestic market, many US industries are finding themselves being undermined. At present an enormous nationwide effort is in train to come up with some policy options for President Carter. By contrast the British document a mere 26 pages in length looks slim indeed, and sensible as many of the suggestions are, they sadly lack the sort of detailed analysis, case studies and reference to give the paper the weight which will impress ministers and their advisers.

The report lists, as obstacles to innovation in Britain, continual changes in the economic environment, lack of incentives for managers and entrepreneurs, the burden of legislation, overmanning, falling industrial productivity, emphasis on short term returns, lack of qualified manpower and technological ignorance in board rooms. The working party, in response to this, recommends a variety of corrective actions—a tax structure more favourable to investment and to small businesses, more encouragement to Research Associations, strengthening university/industry relationships, pressure on nationalised industries and government procurement agencies to support their suppliers, notably small technologically based industries, and so on.

Few would argue either with the analysis, brief as it is, or that the measures suggested were inappropriate, but even so the working party has concentrated its attention heavily on tactics, rather than on strategy. We urgently need to know the answer to questions such as what sort of industrialised nation does Britain wish to become; whether it will incorporate into an industrial policy any considerations of technological interaction with the developing world; whether our present educational system, at secondary as well as at tertiary levels, does enough to make students aware of the importance of less-glamorous industry to our survival as a trading nation; whether there are important environmental, 'public-interest' and health-and-safety issues which ought to be raised in any discussion on industrial improvement; whether there is a 'rational' level at which Britain should spend on R & D.

ACARD have made a useful modest step forward in confronting the question of industrial innovation. It is to be hoped that they will not regard the job as finished. □

Science has a word for it: We recently asked for examples of words or phrases from the world of science that could be given a much wider currency. Some outrageous examples were received of the misuse of words: from Stonybrook came a memorandum describing the university as a 'multi-ethnic and bi-sexual community'; from NASA came 'inhibit time advance' as an instruction to stop a computer programme, and 'versatilify'. But the prize of £10 goes to Donald Higby, of Roswell Park Memorial Institute, Buffalo, for a string of terms from cancer medicine including 'metastazise' as in 'I will have to metastasize to the conference' and 'dose-limiting toxicity' in 'I've reached the dose-limiting toxicity in my relation with Dr X'.





Italian Commission covered up Séveso delays

Italy's official report on the Séveso accident leaves out some important information, writes **Alastair Hay**

THE 412 page Italian Parliamentary Commission Report on the accident at Séveso in Italy in July 1976—an accident in which tetrachlorodibenzo-dioxin (dioxin) from ICMESA's trichlorophenol reactor was discharged over a populated area—should have been the definitive report on the episode. But documents in *Nature's* possession indicate that the claims of Givaudan—a subsidiary of F. Hoffmann la Roche and the owner of the reactor—are more accurate than those of the commission's report.

The bitter and continuing disagreement centres on the sequence of events between 10 July 1976, when the Séveso accident occurred and 24 July when evacuation of the dioxin-contaminated area was ordered. Givaudan claims that it was responsible for urging the authorities to evacuate the area. But the commission's report assigns a far more important role to Italian scientists in recognising the danger. Another report however, that of the Lombardy Regional Government supports Givaudan's claims and indicates that the Italian Parliamentary Commission omitted some vital evidence in its report.

Few would disagree that the two-week delay in ordering the evacuation of residents may have had serious consequences for health. The Italian authorities claim that the delay was the result of a lack of information on the extent of the dioxin contamination. According to the Parliamentary Commission report it was not until the evening of 23/24 July that "positive evidence of the presence" of dioxin was obtained from the results of analyses performed by the Italian laboratories—the Superior Health Institute in Rome and the Provincial Laboratory of Hygiene and Prophylaxis (PLHP) in Milan.

The full version of the commission's report—only recently made available

to interested parties—differs substantially from the summary made available to the press by the Italian authorities when the report was presented to the Italian Parliament in June 1978. The press version implies that the Italian laboratories found dioxin and that their results were used as the basis for ordering the evacuation.

The account in the full report, however, says that two Italian scientists, Drs Ghetti and Cavalloro, flew to Givaudan's laboratory at Dubendorf, near Zurich on 19 July and there received confirmation that dioxin had been discharged into the atmosphere at Séveso. They immediately returned to Italy and duly informed the various authorities. Meetings were held in the days following, and arrangements made for monitoring the situation. It was not until a meeting on 24 July, under the chairmanship of the Health Assessor Dr Rivolta, that the evacuation was ordered. The Italian laboratory results were presented at the meeting, says the report, and confirmed by the Givaudan representative, Dr Vaterlaus.

However, neither the commission nor the press release versions are supported by official reports of the Health Assessor's Council (HAC) of the Regional Board of the Lombardy Region. One controversial issue is the question of whether the ICMESA management notified the appropriate authorities about the accident and its potential seriousness on 10 July. The main HAC report says that it did. All reports confirm that it was the 15 July when the first order was posted by the local mayor to warn the population about the contamination and banning the "consumption of fruit and vegetable products" grown in the vicinity of the factory.

On 18 July, says the HAC report Dr Cavalloro, Director of the PLHP in Milan, suspected the presence of dioxin in the discharge from the

reactor. His suspicion was confirmed by ICMESA although the parent company, Givaudan, says the report, "does not admit that there is dioxin; it allows this to be understood". Under the entry for 20 July, the HAC report says that on the previous evening Drs Cavalloro and Ghetti, during their visit to Givaudan's research laboratories outside Zurich, received confirmation from the company of the "qualitative presence of TCDD [dioxin] outside the factory". *Nature* also possesses the data that Givaudan claims was provided to both scientists. It consists of a map outlining an area of dioxin contamination, based on the results of analyses of some 36 environmental samples.

For the next two days, the 21 and 22 July, both the Parliamentary Commission and Lombardy Region reports are in agreement. However, on the 23 July they differ substantially. The HAC report assigns a greater involvement to Givaudan and Roche in the Lombardy Regional Government's deliberations than does the commission's report. It says that on 23 July a representative of Roche arrived at an extraordinary meeting of the Provincial Health Council in Milan where various Italian scientists with an interest in Séveso were considering



Child exposed to dioxin

the measures to be adopted and was forbidden to speak. Later that day at another meeting—of the local administrators of the Séveso region—the representative, Dr Guiseppe Reggiani, Director of Clinical Research for Roche, stressed to the Health Assessor, Dr Rivolta, the necessity and urgency of evacuating the population at Séveso exposed to the highest concentrations of dioxin. Rivolta asked him to put his concern in writing. The report says that this request was complied with on 24 July in a letter (signed by Givaudan's president, Dr Guy Waldwogel). At the same time, the report adds, a map of the polluted area was handed over, with the first quantitative data on the pollution released by Givaudan. The map contained the results of analyses of 44 environmental samples. The Lombardy Region report suggests therefore, that Givaudan and Roche played a far larger role in documenting the pollution at Séveso than the Parliamentary Commission gives them credit for.

On the 19 July however, Givaudan claims that the two Italian scientists Ghetti and Cavalloro, who visited the company's laboratory near Zurich, received most of this data—albeit from some 36 environmental samples. If this is true why does the Parliamentary Commission report not mention it? (*Nature* has ascertained that this information was presented to the commission during a six hour meeting) and why had the Italian authorities not even considered an evacuation by 23 July when they knew of the dioxin contamination on 20 July?

The Parliamentary Commission report admits that the laboratory facilities and expertise required to assess dioxin contamination at Séveso were not available in Italy. This is one reason why Ghetti and Cavalloro were provided with the necessary dioxin standards when they visited Givaudan on 19 July. Dr Cavalloro declined to tell *Nature* the number of measurements his laboratory had made between 20 July and 11.00 p.m. on 23 July. Asked about the differences between the reports of the Parliamentary Commission and Lombardy Region he felt he was not competent to answer and that as it was a political question it should be directed to the Lombardy Regional Government. He and his colleagues, he said, were "only technicians".

Signor Cesare Golfari, President of the Lombardy Regional Government, told *Nature* that he thought the Parliamentary Commission Report was "good for the Regional Government". In his opinion, it gave an adequate account of events at Séveso. Roche, too, welcomes the overall objectivity of the commission's report—the commission

was equally scathing of Roche, Givaudan and many of the Italian authorities. A spokesman for Roche, however, described the commission's report as telling a "twisted tale". He said that he "certainly" knew that the commission's charge that Givaudan had not informed its workers at ICMESA about the hazards of dioxin was true. He also said that all but 6 or 8 of some 6,000 civil claims for damages against Roche had been settled and conceded that the lawyers had recommended prompt settlement of claims to avoid a higher bill if the criminal court case went against the company.

The criminal prosecution is in the hands of the Italian Judiciary. The court will consider whether or not Givaudan was negligent in its main-

tenance of the trichlorophenol reactor at Séveso, and whether it could have informed people more quickly after the accident of the danger they faced. The hearing is unlikely to take place for some time however, for the judge originally appointed to deal with the case has recently been promoted and his successor will need some time to familiarise himself with the background to the case.

Until the court hearings are available, the Parliamentary Commission report will remain the most comprehensive appraisal of the accident at Séveso. Britain's Health and Safety Executive (HSE), is to have the document translated. When that is available much of what happened at Séveso will be available for public scrutiny outside Italy. □

Genetic manipulation: Britain may exempt "self-cloning"

Britain's Genetic Manipulation Advisory Group will meet this week to reconsider issues which have been bothering the group for some time—particularly the definition of genetic manipulation and whether it should include "self-cloning" experiments.

These are experiments in which the genes of a bacterium are cloned within the bacterium itself—such as *E. coli* within *E. coli*—which are already exempted from control under the revised US National Institutes of Health guidelines.

GMAG has been under considerable pressure from bacteriologists—and lately the Royal Society (see *Nature*, 15 February, page 509)—to relax its control of such experiments, which form a substantial proportion of potential recombinant DNA research; and now it appears to be responding. Even if GMAG decides in favour of a strict definition of recombinant DNA experiments which includes self-cloning, it is felt that it will move to exempt the experiments from categorisation.

At its last meeting GMAG decided "in principle" to adopt the new risk assessment scheme devised by Sydney Brenner—but only for a trial period of about one year. Four proposals for experiments have already been received for consideration by risk assessment, including one application for downgrading, and two have already been assessed. In these cases the categorisation was the same as it would have been under the phylogenetic Williams guidelines, so there was no conflict, but members of GMAG are known to be worried about cases where there is

disagreement. It is felt that much is yet to be learned about real risk factors, and that much more research on them needs to be undertaken before risk assessment can be firmly established.

Meanwhile the "technical panel"—which will make the assessments and advise the Medical Research Council and Health and Safety Executive on risk research that needs to be undertaken—is being established under the chairmanship of Professor Peter Walker of the University of Edinburgh, a man who has been not uncritical of excessive control of recombinant DNA research.

The technical panel will have some nine "core" members, including representatives of GMAG and outside experts, and will also co-opt special expertise when necessary.

This week's meeting of GMAG will be a particularly interesting one for it may be the first at which the deeper views of its seven new members (out of a total of 19) will be tested. The last meeting was concerned with a detailed discussion of the risk assessment scheme, and like many of the regular GMAG meetings it failed to address—or at least reach decisions on—certain matters of principle such as the basic definition of its area of competence. Hence this week's extraordinary meeting, called specifically to address these questions.

If the meeting reaches its conclusions rapidly, GMAG will publish a short document for researchers outlining its new policy. A full note will be issued in due course.

Robert Walgate

MPs accuse health officials of unconcern over risks and benefits of DNA research

BRITISH members of Parliament last week accused officials of the Department of Health and Social Security of displaying an "active disinterest" (*sic*) in the possible health risks of research involving the use of recombinant DNA techniques.

In particular, the MPs expressed concern that a non-specialist assessor who sits on the Genetic Manipulation Advisory Group on behalf of the DHSS should represent the UK on an ad hoc group of experts which has drawn up a draft directive on the regulation of genetic manipulation experiments for the European Economic Commission.

These concerns were voiced at a meeting last week of a subcommittee of the House of Common Select Committee on Science and Technology which is currently holding a series of public meetings on genetic engineering in the UK.

Mr C. M. Regan, under-secretary responsible for public and environmental health at the DHSS, told the MPs that because recombinant DNA techniques essentially involved research processes, their safety was a matter for the Department of Education and Science and the Health and Safety Executive rather than DHSS. Although

DHSS had an assessor on GMAG, the public health aspects of possible hazards was therefore "not a matter which falls to us directly".

The MPs accused the DHSS officials of taking an unimaginative approach, both to their responsibilities for protecting the public from unpredictable hazards of the research, and to the possibilities that the research promised for the production of drugs such as insulin.

Mr Ted Leadbitter, who had last week accused DES officials of using "under-secretary language" in giving evasive replies to the members' questions, referred to the memorandum submitted to the subcommittee by the DHSS as a "pathetic document" revealing what he called an "active disinterest" in the matters under review.

Mr Leadbitter's wrath descended in particular on Dr T. J. B. Geffen, a senior principal medical officer at DHSS who represents the department on GMAG, but who told the committee that he has no particular experience or training in the techniques being discussed, and that his role was essentially that of providing a channel of communication between the department and GMAG.

Dr. Geffen, who was a hospital

physician before joining the DHSS, told the MPs that he did not feel this role required any particular expertise, and that he felt there was no need for the DHSS to employ anyone with such experience since advice on technical matters relating to advances in DNA research could be obtained from consultants.

Given Dr Geffen's "relatively small" contribution to GMAG, the MPs therefore wanted to know why he had been chosen to represent the UK on an expert committee which had prepared draft directives on genetic manipulation for the EEC. They were not satisfied with the answer that the word "expert" in such a context meant someone with broad experience in the matter under discussion.

Mr. Arthur Palmer, chairman of the select committee, said that he felt the DHSS was taking a negative approach to the question of both the risks and the benefits of recombinant DNA research, and that the committee would try to put questions to Mr David Ennals, Secretary of State for Health, "to see if this policy of detachment is the best one".

David Dickson

Forest destruction must be halted, says report

THE Earth's forests are now decreasing in size annually by at least 11 million hectares—the area of Bulgaria—according to the latest Worldwatch report. It warns that man's destruction of tropical forests "is taking an enormous economic and environmental toll".

The study's author, Erik Eckholm, points out that the "fundamental importance of forests to human well-being" is generally ignored by those who analyse the basic needs of the world's poor to reduce the forest deprivation now suffered by many". (The Worldwatch Institute is doing its best to cure the blindness: the very first Worldwatch paper was Eckholm's 'The Other Energy Crisis: Firewood'.)

Information about the extent and condition of the world's forests is remarkably scanty, government statistics are frequently inaccurate and "sometimes doctored outright", the report says. But Eckholm estimates that approximately 20% of the Earth's land is now covered by dense forest—that is



about half of the area that would be forested in the absence of man. Another 10% of land is "open woodland".

The forest area of North America and Europe is roughly stable. Almost all the loss is occurring in the humid tropical forests of Latin America, Asia and Africa, where Eckholm says the crude deforestation figures greatly underestimate the damage because "they reflect neither the massive degradation of timber and other biological resources occurring within many still-standing forests nor the severe depletion of open woodland and countryside vegetation that currently blights most third world countries".

The three principal causes of deforestation are, in order of importance, the unplanned spread of agriculture,

firewood collection for cooking, and indiscriminate timber harvesting. Tree planting makes up only a small fraction of the loss.

"As a result the real prices of wood and wood products are likely to rise substantially over the coming decades . . .", Eckholm writes. "Meanwhile the continued loss of forests will accentuate the environmental costs of denudation already apparent in many countries—erosion, desertification, siltation, flooding and the extinction of species." And, he adds, deforestation may rival fossil-fuel burning as a source of atmospheric carbon dioxide.

Although evidence for the economic consequences of deforestation is extremely scarce, Eckholm argues that it has been a major driving force behind the past decade's worldwide inflation. The price of wood has been rising in real terms by 4% a year during the 1970's, he estimates—and the increases have been most severe in the third world.

"A simple board costs twice as much in Pakistan as in the United States though the income of the average American is 46 times that of the average Pakistani", Eckholm reported. Families in some West African cities spend 1/3 of their income on firewood.

But the study concludes on an optimistic note. International aid agencies and politicians in developing countries are becoming more aware of the need to protect and manage forests. And the dismal record of tree planting in the third world—millions of young trees uprooted or nibbled to death by livestock—is showing signs of improvement in places where the “community forestry” approach is adopted. This

means giving villagers the means to meet their own requirements and protect their own growing trees, Eckholm says, rather than imposing planting programmes in which they have no say—as has happened too often in the past.

The astonishing success of forestry in post-revolutionary China is the best known example of this philosophy. But Eckholm says the “success story of the 1970’s” is South Korea. There a

strong national commitment, combined with new village-level forestry associations, has covered one-third of the country with trees in the past decade and nearly solved an acute fire-wood crisis.

Worldwatch Paper 26, ‘Planting for the Future: Forestry for Human Needs’, is published by the Worldwatch Institute, Washington DC, at \$2.

A day in the life of Yuri Orlov

$$\begin{aligned} & \text{Векторы } \vec{E}, \vec{H}, \vec{B} \text{ и } \vec{A} \text{ в декартовой системе координат } (x, y, z) \\ & (1) \vec{E} = E_x \vec{e}_x + E_y \vec{e}_y + E_z \vec{e}_z \\ & (2) \vec{H} = H_x \vec{e}_x + H_y \vec{e}_y + H_z \vec{e}_z \\ & (3) \vec{B} = B_x \vec{e}_x + B_y \vec{e}_y + B_z \vec{e}_z \\ & (4) \vec{A} = A_x \vec{e}_x + A_y \vec{e}_y + A_z \vec{e}_z \\ & \text{Связи между компонентами векторов} \\ & (5) E_x = -\frac{1}{c} \frac{\partial \phi}{\partial t}, \quad E_y = -\frac{1}{c} \frac{\partial \phi}{\partial t} \cos \alpha, \quad E_z = -\frac{1}{c} \frac{\partial \phi}{\partial t} \sin \alpha \\ & (6) H_x = \frac{1}{c} \frac{\partial \phi}{\partial t} \sin \alpha, \quad H_y = \frac{1}{c} \frac{\partial \phi}{\partial t} \cos \alpha, \quad H_z = 0 \\ & (7) B_x = \frac{1}{c} \frac{\partial \phi}{\partial t} \sin \alpha, \quad B_y = \frac{1}{c} \frac{\partial \phi}{\partial t} \cos \alpha, \quad B_z = 0 \\ & (8) A_x = \frac{1}{c} \frac{\partial \phi}{\partial t} \sin \alpha, \quad A_y = \frac{1}{c} \frac{\partial \phi}{\partial t} \cos \alpha, \quad A_z = 0 \\ & \text{где } \alpha \text{ — угол между вектором } \vec{A} \text{ и осью } OX \\ & \text{и } \phi \text{ — потенциал} \end{aligned}$$

A jailed dissident is keeping up his scientific work in a Soviet labour camp.
Vera Rich reports

Yurii Orlov, founder-member of the Moscow Helsinki monitoring group, was arrested two years ago this month. Unlike Academician Andrei Sakharov, who has virtually given up scientific work to devote himself to human rights, Dr Orlov, until the moment of his arrest, considered himself a scientist. Indeed, his letters from prison (which reached *Nature* recently) indicate that he is still attempting to pursue his research.

Until his trial last May, Orlov was held incommunicado. After his trial he was permitted to write the usual two letters per month, and immediately began to request writing materials and scientific texts. “I’d like to have Landau’s quantum mechanics”, he wrote to his wife in July, “it’s lying on the shelf”.

In the same letter he reports on his work in progress. “I’m constantly doing scientific calculations. . . . As you know, I’ve already completely finished one paper on logic, when I’ve copied it out, I shall send it officially to the Landau Institute of Theoretical Physics, to the Director.”

However, two papers written during his pre-trial confinement had been confiscated. The next letter suggests, obliquely, that he was contemplating a hunger-strike if they were not returned.

Three weeks later, he was still anxious about the fate of these papers, and further perturbed that his paper on logic had not reached the director of the Landau Institute. But a long postscript to be passed on to his scientific friends outlined his latest

train of thought. “While I was in quarantine, I did a small calculation for myself. I considered whether it was possible, by passing a beam of light along a wave-guide (i.e. with total internal reflection), to obtain beyond the limits of the wave-guide, in vacuo, an electromagnetic wave extended to a fairly great distance.”

But the conditions for such quiet scientific reflection had already come to an end. In mid-August, Orlov had been transferred to a labour-camp where he found himself doing heavy physical work at an altitude of more than 1,000 m which left him completely incapable of any serious scientific activity.

By mid-October, when he had moved to another camp, No. 37, his letters once again show interest in scientific matters. One letter deals almost entirely with problems of cybernetics and his concept of “wave logic”. There are interesting references to past discussions with Gol’fand, Rozenshtein—presumably within the framework of the refuseniks’ “Sunday seminars” of which Orlov was one of the few non-Jewish members.

But his conditions of work were still those of hard labour. “I’m working in the plant all week now. I had a choice: cutter or turner. The cutting shop makes a great deal of noise; in the turning shop you have to keep spinning around. I chose the turning shop. I’m still a learner: how it is possible to fulfil one’s norm, I still can’t imagine. . . .”

Yurii Orlov has seven years to serve in the labour camp, plus five years “internal exile”. The avowed purpose of work in a labour camp is rehabilitation through service to the community. In almost every respect, conditions for political prisoners have improved considerably in the 25 years since Stalin’s death. The one exception is that of scientists. As Orlov’s friend, who forwarded the letters to *Nature* pointed out: “In Stalin’s time, scientist-prisoners did research in special prison laboratories — Solzhenitsyn’s *First Circle*. They didn’t have to waste their talents in physical labour, as Orlov does”. □

Soviets seek US collaboration over biosatellite

SOVIET space biologists have invited their US opposite numbers to take part in the experimental programme for the next Soviet bio-satellite scheduled for the end of 1979.

This proposed venture was announced by Dr Nikola Gurovskii, a leading member of the Soviet team shortly before a new agreement was signed extending cooperation between the Academies of the two countries. Much of the agreement dealt with details of exchange visits (up to 100 person-months per year) and research visits (16 from each side per year of from 3 to 12 person-month). However, there was also a general acceptance of the desirability of using the new agreement to place greater emphasis on cooperative activities, dealing with important scientific problems—a slot which would neatly take in the bio-satellite.

Dr Gurovskii said that this satellite would include 13 joint experiments. For the first time, the effect of weightlessness on mammals (rats) and birds (quails) would be investigated. There would be further investigations of the physiological effects of space-flight, and also tests on a new “anti-radiation” medicine, using dosimeters manufactured in the US.

These latter aspects are of particular interest to Soviet space medicine, according to a recent article in *Pravda* by Dr Avetik Burnazyian. He stated that the decisive factor limiting space-flights is no longer a technical one, but the ability of the organism to re-adapt to Earth conditions.

The proposed joint biosatellite programme will not be the first time there has been such cooperation; similar though less ambitious joint programmes were carried out on the Kosmos-782 and -936 satellites.

In Dr Gurovskii’s opinion, however, the extension of such cooperation is of major importance. It is becoming ever more difficult, he said, to develop science in a “narrow national framework”. For “truly outstanding results”, international cooperation is essential.

Vera Rich

news in brief

Unions refuse to enter plutonium areas at Aldermaston:

Management at the UK weapons research plant at Aldermaston has tried unsuccessfully to reopen work areas that have been closed since last August when 12 workers were found with excessive plutonium in their lungs. According to a spokesman from the Ministry of Defence the buildings are open in the active areas but restaffing of the buildings "has not been achieved". The MOD says that the issue under negotiation is danger pay but that it would be "inappropriate to disclose the details". According to the *Observer* (18 February) the unions are demanding £10 per day and management is offering 13p per hr. Bob King, shop steward of the Transport and General Workers Union at Aldermaston, the largest of the four unions involved, says the issue is not danger pay. "We've never said that. It's a term adopted by the newspapers." The TGWU is demanding that the area be made safe before they will allow their members to work in it. The unions will rely on health specialists in their national offices as well as local staff experience to insure that reasonable safety standards are met.

KGB clamps down on Sunday seminar: Reports from Moscow indicate new KGB pressure against the Sunday scientific seminar for refuseniks. It is feared that the authorities may be trying to stamp out the seminar altogether—perhaps before the Einstein centenary next month focuses attention on the Jewish contribution to science.

A criminal file, No. 41035/38-75, has been opened on Viktor Brailovskii, the seminar leader, and a certain Avram Yoffe, from Leningrad. One of the people questioned in connection with this file was told by the interrogators that charges were being formulated and a trial could be expected "quite soon". One disturbing fact is that the investigations have been extended to the non-refuseniks who have been attending the seminar.

Budget cuts threaten London medical schools: Lord Annan, Vice Chancellor of London University has set up a working party to investigate the financial viability of the University's 12 general medical schools, five dental schools and 13 postgraduate teaching and research institutes. Chaired by Sir Brian Flowers, Rector of Imperial College, the working party is to investigate the effects of further reductions in funds to the university and will prepare plans for the closing of specific schools if it finds this necessary. The action was taken after the Joint Medical Advisory Committee heard numerous complaints that budget cuts were impairing the quality of training. The university trains 30% of British doctors. In 1977/1978 8,250 medical and dental students were enrolled, of which 6,700 were undergraduates and 1,550 were postgraduates.

UK to build amateur spacecraft:

Amateur radio enthusiasts at the University of Surrey are to build the UK's first amateur spacecraft—a satellite carrying a series of high frequency beacons which will be used by radio amateurs to study the effects of the ionosphere on radio-wave propagation. It is to be built in close collaboration with the international Amateur Satellite Corporation (AMSAT), the Amateur Satellite Organisation of the UK (AMSAT-UK) and the Radio

Society of Great Britain. At an estimated cost of £150,000, the satellite should take two years to build. It is expected to be launched early in 1981 into a polar orbit at a height of 900 km. As all of AMSAT's previous eight amateur satellites have been launched by NASA, it is likely that NASA will be approached to launch this one too. The one exception, however, is the German-built AMSAT satellite which is due for launch by Ariane.

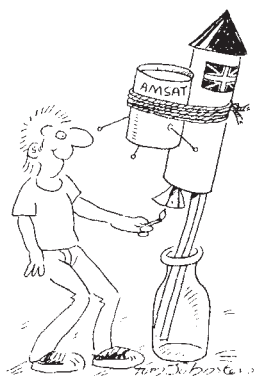
Swiss nuclear safety referendum result: Strict nuclear reactor controls (*Nature* 15 February, page 508) were rejected by the Swiss electorate on 18 February by a vote of 265,271 to 919,217. Nine cantons voted for the referendum and 13 against it. The 49% vote in favour is considered to be a surprisingly good result for the nuclear opposition since the nuclear industry spent 20 million francs on its campaign. So says André Froidevaux, of the National Coordinating Committee of Swiss Anti-Nuclear Organisations: "the nuclear lobby successfully exploited working class fears that jobs would be lost if the referendum passed".

An alternative law has been put through parliament. But the anti-nuclear movement has gathered 50,000 signatures to force another national vote on this law on 20 May. The new law gives parliament the right to veto new power stations and requires the need for a station to be documented. There is also a provision giving parliament the right to expropriate land for nuclear waste disposal. This law will be opposed by the National Coordinating Committee on the grounds that it offers virtually no effective controls to reactor development.

Cambridge designers plan low energy homes: A new design of terrace houses that will cut heating costs by 75% from £120/yr to £30/yr has been proposed by the Cambridge based firm, Ambient Energy Design. Their plan, which received first prize at the Energy Show now underway in Birmingham, makes use of better cavity insulation, double glazing units with infrared reflecting film between the panes, roll shades for night time insulation, and a conservatory which will trap solar heat. Gerald Foley of the International Institute for Environment and Development calls the savings "impressive". According to Foley, 30% of primary energy in the UK goes to housing of which two-thirds is for space heating. Thus a cut in heating requirements of 75% means a savings of 15% in the overall energy budget. Members of AED are convinced that low energy consumption represented by the above measures is "economic and realistic and will become commonplace."

Ambient Energy Design—House Heating for the 1980s. 6 Apthorpe Street, Cambridge CB1 5EY, £1.50.

Third World think tank proposed: S. S. Ramphal, Commonwealth Secretary-General has proposed to the South-North Symposium held in Tanzania last December that a Geneva-based research organisation be created to back up a Third World secretariat that can be competitive with existing organisational alliances of the developed world. In 1977 there were over 2,000 meeting days for UNCTAD alone and only 56 of 117 members of the Group of 77 developing countries had missions there. In comparison, organisations like OECD, EEC, and NATO exist to provide cohesion amongst the developed countries. Ramphal proposes that an OECDC, Organisation for Economic Cooperation among Developing Countries, be formed. The OECDC will "of necessity, be technocratic and multinational". Technological improvements in data storage, retrieval and information processing are necessary for the South to negotiate successfully with the North.



US report fails to bridge gap with Third World

The US delegation's paper for the UNCSTD conference in August is a disappointing document, writes **Ward Morehouse**

The long-awaited US National Paper for the UN Conference on Science and Technology for Development has made its appearance more than five months after the UN deadline for submission of national papers. It is strong on diagnosis, but woefully weak on remedies for the economic and social ills of developing countries which are identified in the paper; and unless the US is markedly more forthcoming at Vienna, this document will slip into the oblivion of history as yet another contribution toward the widening gap between profession and performance by industrialised countries toward the Third World.

Let us start with what is right in the report. There is recognition that the transfer of proprietary industrial technology has sometimes had a polarising impact on Third World economies; and that the deals struck by multinational corporations may not have always been fair to developing countries. Indeed the US paper urges that steps be taken to strengthen the negotiating skills of Third World countries on the grounds that more equitable bargains are likely to be more durable.

And the US report rightly recognises the failure of development strategies seeking solely to maximise growth. (Though its assertion that "the 'trickle down' strategy is now dead" taxes the indulgence of many observers.)

There is even recognition of the vital importance of building a strong science base in the Third World and an acknowledgement that most development assistance, focussed on short-term projects with immediate impact, "did not alter developing countries' continuing dependence on the initiatives of the scientific centres of the Northern Hemisphere". In a similar vein the report recognises "the need for developing countries to establish their own policy framework and to set their own priorities", although the report freely indulges in the imperialism of social priorities by repeatedly asserting the primacy in US development assistance of meeting basic human needs.

But the outstanding omission in the report is its failure to reflect the ambivalence of US policy toward the Third World.

In view of the lacklustre and self-serving character of most (but not all) of the other industrialised country

national papers for UNCSTD, searching political self-analysis may be too much to expect. But the promising effort to analyse the economic and social shortcomings of US science and technology and other development policies stands in sharp contrast to the lack of political analysis.

While the US and other industrialised countries would no doubt like to see the worst forms of human poverty eradicated and a "self-generating" process of equitable economic growth accelerated in the Third World in the hope of reducing the possibility of overt and violent conflict between North and South, they are also interested in maintaining or extending political and economic dominance in the international system.

This dominance will give them an assurance of continued access to export markets and raw materials in developing countries, thereby helping to retain and enhance their relatively affluent living standards. And as other forms of economic and military power decline, technology is becoming an increasingly attractive and potent instrument for maintaining that dominance. President Carter's National Security Adviser, Zbigniew Brzezinski, has said that "the emergence of more advanced Third World countries like Brazil and Korea as new centres of rapid industrial growth means that the older industrial countries have to rely increasingly on technological innovation to maintain their place in the world."

Therefore it is hardly surprising that the US paper makes no direct reference to Third World efforts to change the rules of the game—for example, the proposed code of conduct on transfer of technology being negotiated in UNCTAD or the forthcoming conference to consider revision in the Paris Convention on patents.

The report even asserts that "the US Government has been generally neutral toward commercial transactions of US private firms" ignoring the strong political support in international negotiations the government gives to positions which seek to maintain the *status quo* in international technology and investment flows and which serve well the interests of US multinationals. Only the scantest reference is given to the powerful influence which US tax, investment, tariff and trade policies have on these flows.

Likewise the report gives little recognition to powerful domestic political and economic interests which shape

US development and trade policy toward the Third World—as in the attempt by adversely affected US producers of vegetable oil and other products to prohibit use of US contributions to the World Bank and other multilateral financial institutions for projects designed to increase output of these products in developing countries.

The centrepiece of the US response to these problems is the proposed Foundation for International Technological Cooperation, although it is well known in Washington that the Foundation faces stiff opposition to its creation in Congress and even within the Executive Branch and may never come into being, let alone with the degree of autonomy and magnitude of resources to make a real difference in overcoming the failures of past policy and performance. All in all, it is pretty mild therapy for such a serious set of maladies as identified elsewhere in the US national paper.

The greatest defect of all in the US paper is a failure to recognise that the industrialised countries, and the science and technology on which those countries have a near monopoly, are as much a part of the problem as they are of the solution to the ills of the Third World. The insatiable demand for consumer goods by the rich minority of the world's population has generated technologies which are displacing millions of previously independent farmers and artisans in the Third World from their livelihood.

In the words of the Poona statement calling for open debate at UNCSTD on science, technology and the human condition in the modern world: "They would be players in the theatre of the absurd if they did not occupy positions of power and influence in the real world of today. Humanity must regain its sanity by reasserting the nature of scientific inquiry as the pursuit of truth, leading to technologies which liberate the many rather than facilitate control by the few." □

● Last week, President Carter announced the expected reorganisation of the US foreign aid programme. A new International Development Cooperation Administration (IDCA) will administer 2.7 billion dollars of non-military assistance programmes currently controlled by the Agency for International Development. IDCA will include the proposed Foundation for International Technological Cooperation (*Nature*, 25 January) but will be only semi-independent. The State Department and the Treasury will retain ultimate control of any major policy decisions. □

Ward Morehouse is president of the Council on International and Public Affairs, and teaches at Columbia University.



Nuclear Energy: how the odds are stacked against its opponents



● **PETER TAYLOR** explains why he feels that risk assessment is inadequately incorporated into democratic decision making in the UK.

THE debate over the safety of nuclear reactors illustrates a general problem of dealing with the impact of a technology, namely that the issues are assumed to be primarily 'scientific' and 'technical', and thus to be the exclusive field of 'experts'. Where procedures have been set up to make energy policy decisions or recommendations in the light of this controversy—as at the Windscale public inquiry—expert witnesses have held the arena and a judge appointed to sort out the "facts" and the "truth". At Windscale all witnesses were sworn to "tell the truth, the whole truth, and nothing but the truth". But as Dr Brian Wynne, who gave evidence at the inquiry, said later: "You can expect a scientist to tell the truth, and nothing but the truth, but not the whole truth."

At the inquiry, Wynne had set out to show how the nuclear controversy differed from true scientific debate. The guarantor of truth in science, he argued, was pluralism in research, availability of information and open critical debate; but the nuclear debate was clouded with secrecy in crucial areas, and critics had totally inadequate resources to match proponents. In safety assessment, for example, there was only one body of facts according to one set of assumptions. Furthermore, scientists within the nuclear institutions, who might wish to dissent, were rigidly controlled by management and by the Official Secrets Act.

The possibility of a major escape of radioactive particles, however much it may now be dubbed incredible by the operators, has influenced siting policy. The early Magnox reactors were all built well away from conurbations. Then, in the late 1960s, pressure mounted to site new reactors near load

centres and a review of siting policy took place. The Nuclear Safety Advisory Committee reported that "the major contribution to public safety lies in the standards achieved by design, construction, and operation of the plant". The Ministry of Power then reported to Parliament on 6 February, 1968, that the new advanced gas cooled reactor (AGR) could be operated "nearer to built up areas than we have so far permitted".

At the time of this policy change, the most publicised aspect was the inherently safer design of the AGR, using pre-stressed concrete for the pressure vessel and internal boilers. However, it was also realised that a large release would have a huge impact, however remote the siting. How far this affected siting policy is not clear.

This period of nuclear development was essentially uncontroversial. Assessments of risk were carried out within the nuclear establishment and accepted as adequate by politicians or closed working groups. It was during this period that risk assessment evolved the technique of "probabilistic analysis". Reg Farmer, at the United Kingdom Atomic Energy Authority, developed it as an aid to the designer in deciding what degrees of redundancy and containment might be reasonable to incorporate. He sought target probabilities of failure and the consequences and compared them with other industrial hazards.

The method has its limitations and, to his credit, Farmer has been quick to caution the nuclear industry for taking too much credit for a few hundred reactor-years of safety, and for "failing to see or adequately have regard to all those minor and sometimes major features of equipment or of organisation which might nearly have led to disaster".

Unfortunately this caution has not been repeated by some public advocates of nuclear expansion. The target probabilities of Farmer's curves of fre-

quency against magnitude of effect (in deaths) have acquired a reality comparable with the facts of meteorite impact, and the frequency of dam failures, aircraft crash, and so on. For Sir John Hill, chairman of the UKAEA, such curves "show the probability of . . .", and for Sir St John Elstub (together with various presidents and vice-presidents of certain engineering and chemical institutes in a letter to *The Times*) the fact that Professor Rasmussen "has calculated" the chances of a severe accident for 100 power plants at one incident in 100,000 years is adequate assurance (a conclusion since repudiated by the US Nuclear Regulatory Commission).

The Flowers report served in some way to redress the balance and introduce critical comment into the public arena. The commission noted that in probability analysis and attendant fault-trees not all failures could be foreseen nor all components ascribed a frequency of failure. They concluded: "Whether the safety performance is achieved in practice will depend to some degree on factors assumed in the analysis, such as the quality of maintenance".

Despite this critical attitude to assessment, they were led to conclude that estimates of damage following an accident "must be presented in probabilistic terms". But, in our view, probabilistic presentation obfuscates rather than illustrates the potential effects. A range of incidents under specific conditions of wind and weather, including the worst of such conditions, should be presented. Probabilities are often meaningless: the most that can be said is that the worst case is less likely than the others.

The argument against worst case site-specific studies has always been that when disasters have occurred, the worst has not happened. After the Amoco Cadiz we can expect that this is not an argument that will be repeated for oil slicks. And yet, there is still a

reluctance to publicise the worst that can happen at Canvey Island or Hinckley Point. We suspect that Justice Parker's attitude prevails: in view of the (accepted) probabilities, such studies would "unnecessarily alarm the public".

That the public might be alarmed is evident when one considers that Flowers' example of a reactor (AGR) accident's consequences, and the NRPB/NII Breeder study, both admit that, under adverse weather conditions, casualties might be ten times worse: in the former case 100,000 casualties; in the latter 600,000.

There is a need for publicly available site-specific studies for AGRs and FBRs (fast breeder reactors) that explore the whole range of potential accidents and which do not slavishly accept the probability calculations of the nuclear establishment. In this, Britain lags far behind the United States and some European neighbours. In the US there have been open critical reviews of risk assessment, aided by the leaking of internal reports, the defection of industry-based scientists, the existence of the Union of Concerned Scientists and the operation of a Freedom of Information Bill. There has thus been an open reassessment of the work of closed expert groups. The most forthright report is that of the American Physical Society's study group on Light Water Reactor safety.

This pattern has been repeated in Sweden, Denmark and Holland, Austria and West Germany, often with the governments funding critical experts. In some cases, dissenting reports have been published by the government printer and incorporated into the procedures leading up to energy policy decisions. Most recently, in West Germany, a panel of 20 leading international, critical experts has been commissioned to review the proposal for reprocessing at Gorleben.

But in the UK, there has been no major reassessment of the work of closed expert groups. Critics are expected to fund their own safety analyses, without research facilities and without access to internal reports on which to base assessments.

Two examples from the work of our group may serve to illustrate the need for independent assessment.

● When the NRPB (National Radiological Protection Board) fast reactor study was published, press comment was largely restricted to assertions of probabilities such as 1:1m, and of the worst case being 60,000 casualties. Having been given access, at Justice Parker's instruction, to the UKAEA computer programme, we were able to run the NRPB calculations (which were for a notional site) for a specific locality (we chose Kalkar in West Ger-

many). We discovered that the NRPB had chosen not to present their own worst calculations—the results of one weather category which led to 600,000 casualties. Our site-specific study, having altered only the population data on the programme, produced over 900,000 casualties.

● Hinckley Point has already suffered a potentially severe "loss of coolant" incident. A pipe-break and simultaneous valve failure led to the loss of both primary and secondary cooling. Dr Franklin of the Nuclear Power Company, when asked at Oxford recently about the ascribed probabilities of these events, said he thought cooling had been restored almost immediately through the use of fire-hose fittings. This statement did not accord with that of the Energy Minister in a written parliamentary answer, where we learn that cooling was lost for three hours and that fire-hose fittings were not present on that mark of AGR.

The scale of concern may be judged from the following extract from the proceedings of a Symposium in Vienna in 1967: "Cave and Holmes of the CEBG gave a probability of failure for primary and secondary cooling of 1:100,000 per reactor lifetime per independent system. Furthermore they praised the inherent safety of the AGR (over the LWR), in a loss of coolant situation, in that the heat capacity of the boilers, etc., was such that there was a period of three to four hours before the fuel cans would melt."

In the light of this time period, the Energy Minister's comment that there was no danger of a release during the period of loss of cooling takes on a new meaning.

Our concern at this uncritical acceptance of safety assessment has heightened recently with the public debate on the risks of different types of energy production. The Rasmussen-type probabilities have now been factored in to nuclear safety statistics. Thus one severe accident killing, say, 10,000 people in so-many-thousand years is incorporated into a global deaths-per-Gigawatt-year in the same manner as mining deaths are fed into the coal generating option. This initial work by H. Inhaber of the Atomic Energy Board of Canada finds that the nuclear option is safer than either coal, oil or solar. Lord Rothschild used this study in a televised broadcast on risks of energy production.

But Inhaber's work assumes reactor accident frequencies which may or may not be borne out in practice. This has, unfortunately not inhibited its use in the industry's public relations. The situation is more disquieting considering that the nuclear establishments

have available to them the fruits of an IAEA sponsored research programme on the comparability of risks which clearly highlights the limitations of Inhaber's approach.

The selective use of the results of scientific research, often in an unrefereed form, raises important questions for the future structure of public debate. The problem will arise when considering the forthcoming FBR inquiry. It is our view that AGR and FBR fault analyses will have to be publicly presented and discussed before comparison of energy option risks can be meaningful. If critical experts are to be expected to produce safety analyses, then they will have to have access to official information, if not government support, for their research.

Finally, there is an area of research relevant to safety assessment that is continually neglected, namely the "social facts" relating to the nature of the dispute itself, the role of science, and of controversy. What groups are involved in the debate and what benefits or risks accrue to each (in the nature of contracts, jobs, risk of accident or pollution)? How is risk perceived and evaluated within these groups and what are the components of attitudes? What correlations are there to levels of knowledge of the technology? What procedures exist for debate, participation and information-exchange and how effective have these procedures been in the past?

The controversy itself may bring forth bitter and unresolvable conflict. Once more we find it disquieting that the UKAEA does not choose to publicise the results of further IAEA work in this field. The work of Pahner, Novotny, Stallen and Meertens points clearly to a realisation that the controversy has no resolution that people are divided by a basic evaluatory perception of technology and progress.

We believe that herein lies a fundamental question. The nuclear issue is important as a first testing ground for democratic flexibility. The institutions of industrial growth and technological progress realise that the call by the "alternative" camp for openness, reassessment and participation, however sincere in itself, exposes aspects of their technology which it feels a lay and mistrusting public may not accept. Thus there is a retreat into secrecy and, paradoxically, a massive public information programme. This in itself generates further opposition. We are not convinced that the UK government realises the full impact of this conflict.

Peter Taylor is co-director of the Political Ecology Research Group. This article is based on a talk he gave at a conference on the politics of fast breeders in London at the end of last year. The conference proceedings are to be published by Macmillan.

correspondence

The Piltdown hoax: *cui bono?*

SIR,—By far the most sensational feature of the Piltdown hoax, that has remained hidden from the public gaze for so long, is that it was planned and executed by eminent scientists against one of their own number.

Until recently there have been two main hypotheses regarding the identity of the perpetrator of the Piltdown hoax. That espoused by Weiner (*The Piltdown Hoax*, Oxford University Press, 1955), inferring the guilt of the amateur Dawson, is far the more acceptable as it absolves the academic world from any involvement. The other view is that Dawson was the scapegoat, and the hoax was part of a conspiracy on the part of scientists to delude the public into accepting the anti-religious idea of human evolution (M. Bowden, *Ape-men fact or fallacy?* Sovereign Publications, 1977). This thesis, of which there are hints in Langham's letter (18 January, page 170), even suggests that Smith Woodward "may have been a willing accomplice". Hence, Elliott Smith, who nailed his banner firmly to the Piltdown mast, could be imagined to have been the instigator.

Douglas (2 November, page 11) has provided a signal service by making us look afresh at the evidence and consider a "grudge against one of the principals" as a fruitful line of enquiry. The incident of the Sherborne horse's head, if nothing else, was a successful and deliberate demonstration by Sollas of Woodward's incompetence. In this context, it is indeed difficult to imagine Dawson being party to any conspiracy aimed at belittling his close friend of many years standing (from 1884 until his death in 1916). He may possibly have been persuaded to help "encourage the evidence", and it is difficult to avoid postulating some kind of direct involvement. The way in which the desperately wished for lower jaw was flicked up by Dawson's pick, so that it landed literally at Smith Woodward's feet, has such an air of theatre about it that one is bound to be suspicious of such a startling coincidence. When the search was on for the missing canine, Teilhard de Chardin sat down to rest on a heap of gravel that Dawson and Woodward had already sifted. They actually told him that there was no point in searching there, but within a few minutes of casual probing with his fingers the canine appeared, yet another startling coincidence. (This event might well place Teilhard de Chardin among the suspects). The subsequent sudden appearance of the "cricket bat", from the soil under the hedge where they usually lunched, was surely stretching credulity to breaking point. Everything that Smith Woodward wanted, turned up and, moreover, in the order in which he wanted it.

Every good hoax, to be effective, needs to carry certain hallmarks that

allow it to be recognised as such by all and sundry, with the main exception of the victim for whom it was designed. Let us now look, not at Woodward's friends, but at his enemies. Douglas has already drawn our attention to the strong possibility of Sollas being involved. Sollas was Britain's leading expert on fossil man, who had just given his Presidential Address to the Geological Society on fossil man and had recently published *Ancient Hunters* (1911). Here was a man who had pioneered over many years the technique of serial sectioning, which enabled palaeontologists to examine the internal structures of fossils that otherwise would never have been accessible for study, a technique which Smith Woodward contemptuously dismissed as a "mere toy". And here was Smith Woodward with pretensions, but no expertise, announcing his ambition to discover the earliest man, and moreover, in Britain. Was this not as close to an invitation as one could imagine?

Weiner (4 January, page 10) draws attention to Oakley's letter to *The Times* (7 November 1978) regarding Sollas's possible use of potassium bichromate, but he seems to have missed my reply (*The Times*, 25 November) refuting Oakley. My letter also mentioned that Dr M. A. C. Hinton was the likely source of the medieval orang utan lower jaw and that Hinton had stated that the hoax was initially planned and executed within the Natural History Museum. Unfortunately the short paragraph referring to Hinton's possible involvement was edited out of my original article in *Nature* (2 November, pages 11-13).

In the spring of 1912 Dawson brought the five Piltdown skull fragments to Smith Woodward, but to be certain of its import, it was vital to find the lower jaw. In 1910 Hinton was given the status of a Voluntary Worker in Smith Woodward's Department of Geology to work on fossil rodents. He managed somehow to offend Smith Woodward to such an extent that in 1912 he transferred to the Department of Zoology to continue his work there (eventually in 1936 becoming Keeper of Zoology and retiring in 1945). The available evidence suggests that the medieval orang utan lower jaw came from a box of unregistered material from the Everett Collection to which Hinton then had access. Hinton (*The Times*, 4 December 1953) stressed that the zoologists in the museum would have unhesitatingly referred the lower jaw and canine to a chimpanzee had the material come within the orbit of the Zoology Department! He claimed that in their eyes G. S. Miller's analysis settled the matter and relieved them of the necessity of expressing any opinion and thus arousing hostility within the museum. In retrospect, this epistle from Hinton takes on a new significance. From the people that knew Hinton

well, it is accepted that he must have been involved in the promulgation of the hoax and it is even claimed that he "virtually confessed" to this, prior to his death. Nevertheless, there was always the nagging suspicion that there was a more knowledgeable figure in the background; Douglas's suggestion at last provides the clue to his identity. Douglas asks "Did they [the people who exposed the fraud] ever try to find out if Smith Woodward had any particular enemies who might do such a thing? No. They did not". If a crime is committed against an individual, the first task, having ascertained that the deed has in fact been committed, is to determine if anyone had a strong motive. Such an approach would have pointed in the direction of the Natural History Museum and Oxford. Without wishing to be uncharitable, perhaps this is the reason the grudge theory was never followed up. This would explain the cameo of Sir Gavin de Beer "rolling on his settee, wringing his hands and crying 'What can I do? What can I do?'" as the evidence on Piltdown unfolded. It is not a scandal for an institution to have been duped by a hoax, but if it transpires that it has been initiated in the same institution, it certainly is.

Finally Weiner's accusation of Douglas besmirching Sollas's reputation cannot be left unanswered. Given the circumstances in the 1910-1912 period, given the personality of Smith Woodward, I wonder how many people would have resisted the temptation "to present somebody, who is claiming to be an expert in a field, with something everybody would know is so ludicrous that he would make an utter fool of himself and be shot down in flames". The conspirators misjudged the perspicacity of their colleagues, although Waterston, Lankester, Boule, Miller and Weidenreich doubted the association of ape jaw and human skull from the very beginning. Above all, however, Piltdown engendered such an enthusiastic nationalistic fervour, that the critical faculties of the scientific community seem to have been overwhelmed, and this surely was the tragedy of Piltdown.

Yours faithfully,

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False- or pseudogalena

SIR.—Joe Schwartz (25 January, page 255) has confused galena with false-galena or pseudogalena (sphalerite).

Yours faithfully

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news and views

Pion interferometry of nuclear collisions

from Miklos Gyulassy

THE measurement of the space-time dimensions of nuclear interaction regions formed during high energy (~ 1 GeV per nucleon) nuclear collisions obviously calls for rather novel techniques. That interaction region (which we call the nuclear fireball) may be roughly cigar-shaped with longitudinal and transverse dimensions ranging between 10^{-12} and 10^{-13} cm and expanding with relativistic speeds on a time scale of 10^{-23} to 10^{-22} s. In that tiny, short-lived fireball, nuclear matter may be compressed to very high densities ($\sim 10^{15}$ gm cm $^{-3}$) and heated to enormous temperatures ($\sim 10^{12}$ K).

The physics of nuclear systems under such extreme conditions is now the subject of a new branch of nuclear science known as relativistic nuclear collisions. Recent developments in this area have been reviewed by Goldhaber and Heckman (*A. Rev. Nucl. Part. Sci.* **28**, 161; 1978). One of the most exciting new developments has been the application of pion interferometry to deduce the space-time geometry of that nuclear fireball. The principles behind pion interferometry and the first experimental results are discussed below.

The basic idea goes back to Hanbury-Brown and Twiss (*Nature* **178**, 1046; 1956), who first used the technique of second order intensity interferometry to deduce stellar radii. They made a remarkable observation for the chaotic, incoherent light emitted by stars. While the average intensity (photon count) measured by two detectors A and B was the same, $\langle I_A \rangle = \langle I_B \rangle$, and independent of the separation R_{AB} between the detectors, the average coincidence rate, $\langle I_A I_B \rangle$, did depend on R_{AB} . For large separations $\langle I_A I_B \rangle \approx \langle I_A \rangle \langle I_B \rangle = \langle I_A \rangle^2$, but for small separations, $\langle I_A I_B \rangle > \langle I_A \rangle^2$. From the dependence of $\langle I_A I_B \rangle$ on R_{AB} , Hanbury-Brown and Twiss were able to measure stellar radii.

The reason for this variation of the coincidence rate as a function of R_{AB}

is now well known and found in any modern quantum optics textbook. It originates from Bose-Einstein statistics required for integral spin particles such as photons. It is the interference between the two parts of the symmetrised wavefunctions, $1/\sqrt{2} \{ \psi_1(x)\psi_2(y) + \psi_1(y)\psi_2(x) \}$ describing two identical bosons that leads to the intensity interference. Physically, the enhanced correlation at small R_{AB} follows from the tendency of bosons to congregate in the same state.

Not long after the discovery of the HBT effect in astrophysics, it was rediscovered in high-energy physics in measuring correlations between identical pions. In high-energy physics it is called the GGLP effect (*Phys. Rev.* **120**, 300; 1960). Pions ($\pi^+\pi^-$) are also bosons and are thus subject to the same symmetrisation requirement as are photons. Consequently, intensity interference must also occur for chaotic pion fields. For pions, intensity interferometry involves the comparison of the coincidence rate of identical pions with momenta $\mathbf{k}_1$ and $\mathbf{k}_2$ to the random rate of observing pions with $\mathbf{k}_1$ and $\mathbf{k}_2$. Bose-Einstein statistics lead to an enhancement of the coincidence rate over the random rate for small momentum differences. The correlation function, defined as the ratio of the coincidence to random rate, is thus larger than unity for small $|\mathbf{k}_1 - \mathbf{k}_2|$. According to a theoretical analysis by Kopylov and Podgoretski (*Sov. J. Nucl. Phys.* **18**, 336; 1974), the dependence of the correlation function on $\mathbf{k}_1 - \mathbf{k}_2$ in fact measures the space-time Fourier transform of the pion source density. The relation between the measured pion correlation function and the source density obtained by Kopylov has been the basis of a great many studies of the space-time dimensions of hadronic interaction regions (see for example Enzell *et al. Phys. Rev. Lett.* **38**, 873; 1977).

Most recently the pion correlation data from high-energy nuclear collisions were analysed by a University of California, Riverside group headed by R. T. Poe. The reaction studied was $\text{Ar} + \text{Pb}_3\text{O}_4 \rightarrow \pi^+\pi^- + \text{X}$ using an Ar beam

with 1.8 GeV per nucleon kinetic energy (Fung *et al. Phys. Rev. Lett.* **41**, 1592; 1978). In such reactions often more than 100 charged particles (p, π^+, π^-) are observed with negative pion multiplicities up to 15. These are the remnants of the violent explosion of the nuclear fireball. Pion interferometry has given us the first glimpse of the dimension and lifetime of that fireball before its explosion. The group reported that the average radius, R_0 , and the lifetime, τ_0 , of the fireball were $R_0 = 3.3 \pm 0.9 \times 10^{-13}$ cm and $\tau_0 = 5 \pm (?) \times 10^{-24}$ s.

Although the statistics are as yet too few to determine the longitudinal and transverse dimensions of the fireball separately, the measured R_0 agrees well with our expectations. However, most important, this experiment demonstrated the feasibility of pion interferometry in nuclear collisions. The next generation of experiments with the Bevalac at the Lawrence Berkeley Laboratory are expected to increase the statistics greatly. We will then be able to resolve much more clearly the geometry of these fireballs.

The discussion up to now has been concerned with intensity interferometry when applied to chaotic boson fields. As recently emphasised by Fowler and Weiner (*Phys. Lett.* **70B**, 201; 1977) the intensity interference pattern also depends on the degree of coherence of that boson field. Laser fields, for example, exhibit no intensity interference. The effect of possible coherence on pion interferometry is a topic of current theoretical investigation.

A final comment that must be made is that final state interactions must also be considered in connection with future work on pion interferometry. Unlike photons, pions can interact with each other and the remnants of the exploding fireball. The effects of final state interactions (especially the long-range Coulomb forces) must be unfolded from the measured correlation data before accurate geometrical and dynamical information can be deduced from those correlations. Such studies are also now underway. $\square$

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Exons encode protein functional units

from C. C. F. Blake

An important contribution to relationship between protein structure and the newly discovered mosaic structure of eukaryotic genes, in which coding segments of DNA (exons) alternate with non-coding segments (intervening sequences or introns), is reported by Tonegawa and his colleagues in this week's *Nature* (Sakano *et al.* page 627). The protein chosen was the constant region of the immunoglobulin γ heavy chain. Sequence studies and crystallography have already shown that the constant region is composed of three homologous structural domains: the CH₁, CH₂ and CH₃ domains. Each domain is composed of about 110 amino acids, folded into almost identical tertiary structures made up from seven β -strands. The CH₁ domain pairs with the C_L domain of light chains to form, together with paired V_H and V_L domains, the Fab parts of the γ -globulin molecule. The CH₂ and CH₃ domains pair with equivalent domains of a second heavy chain to form the F_c fragment, which is separated from the Fab fragment by a hinge region. Each of these structures has a well defined function: the paired CH₃ domains act in cell surface interactions, the paired CH₂ domains in complement fixation; the hinge probably transfers information from the Fab to the F_c fragment; the CH₁ pair is the attachment point for the light chain; the V_H V_L pair forms the antigen binding site; and there is also an N-terminal signal peptide. Thus the immunoglobulin molecule is composed of six discrete structural/functional units whose relationship to

the exon/intron mosaic in the coding region could be most illuminating.

The DNA was obtained from mouse MOPC 21 myeloma by complete digestion with *EcoRI* nuclease and the identification of a 6.8 kilobase segment that contained the required sequence by hybridisation with cDNA prepared from MOPC 21 γ_1 mRNA. The segment was recombined with the EK2 vector, phage λ gt WES, and cloned. After digestion of the clone the position of the γ_1 coding region in the 6.8 kb fragment was determined by electron microscopy of the R-loops formed between it and the γ_1 -chain mRNA. This revealed three small R-loops separated by two intervening double-stranded DNA segments, suggesting the presence of three exons interrupted by two introns. A restriction enzyme cleavage map showed that this interpretation was correct, but that there was an additional very short exon located between exons 1 and 2, giving a final pattern of 4 exons separated by 3 introns. Sequence analysis of the intron-exon boundaries and comparison with the known amino acid sequence of the MOPC 21 γ_1 chain, revealed that the exons represent, in sequence, the CH₁ domain, the 14-residue hinge region, the CH₂ domain, and the CH₃ domain. There seem to be no introns within the individual sequences coding for these units.

The conclusion from this study, that each of the six known functional units in the γ -globulin heavy chain is

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coded by a separate exon is most exciting. So clear cut is the correspondence that one is tempted to conclude that the problem of exons has been solved along the lines proposed by Gilbert (*News and Views* 271, 501; 1978) in which the presence of exons was seen as allowing new proteins to be constructed from parts of old ones. The results reported by Tonegawa are exactly in line with this hypothesis, and show that individual exons can define even very small functional units, like the 14-residue hinge region, and not just domains or folding units as I previously suggested (*News and Views* 273, 267; 1978). In view of the precise correlation between exons and the functional units in the immunoglobulins, the presence of three exons in the β -globin gene, when the protein cannot readily be divided into individual units of either structural or functional significance seems at first very puzzling. However, closer examination shows that the central exon corresponds very closely to the protein fragment that defines the haem crevice, and contains nearly all the amino acid residues that interact with the haem. Tonegawa's work implies that exons may reveal unsuspected functional protein units—perhaps like the 'minimal globin' outlined above—even when the joins are not visible in the protein structure. The thought that the exon/intron mosaic may carry the frozen evolutionary history of proteins makes one look forward to see whether further determinations of exon-intron patterns of other proteins of known structure support this view, or point in a different direction.

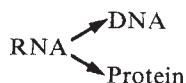
RNA splicing and polynucleotide evolution

from D. Reanney

INFORMATION flow in cells, is defined by the 'central dogma' as

DNA $\rightarrow$ RNA $\rightarrow$ Protein

But although the central dogma accurately describes the flow of information in modern cells, it probably fails to reflect evolutionary perspective. Considered from the chronological viewpoint a more accurate representation of the central dogma might be



This rewriting of a basic tenet of

biology has come from several lines of evidence which strongly reinforce the view of Crick (*J. molec. Biol.* 38, 367; 1968) and others that RNA is the primary genetic material. It is worth re-emphasising that most key features of the translation of genetic information depend on Watson-Crick pairing between (partly) complementary RNAs. Translation is mediated by interactions between different RNAs—mRNA: tRNA; mRNA:rRNA; rRNA:tRNA and rRNA:rRNA. The same was probably true of primitive 'metabolism'. With respect to a metabolic fossil such as the ribodinucleotide NAD, for example, one may ask why is a nucleotide 'handle' a necessary part of a

molecule whose active group is nicotinamide. I suggest that the first steps on the road to precellular metabolism were taken when electron-donating and electron-accepting reactions were coupled by means of molecules whose 'handles' were complementary in the Watson-Crick sense. For example

UDP-X (red) $\rightarrow$ UDP-X (ox)
 where U is paired with A
 NAD (ox) $\rightarrow$ NADH + H (red)

Here the 'genetic' monomers A and U are used to bring the two elements of the redox reaction into appropriate juxtaposition; this is effectively what an enzyme does. The addition of

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further nucleotide 'handles' would enhance not only the efficiency of the mechanism but also increase its specificity. Reaction specificity is another enzymatic attribute; in terms of this thesis, aminoacyl-tRNAs are but one surviving set of examples of a family of oligonucleotides equipped with non-nucleotide active groups.

Perhaps the most compelling argument for a primary role for RNA in early evolution has come from the discovery of RNA processing. At first sight RNA:RNA splicing seems to remove RNA from early genetic events rather than bring it closer to them. This is because splicing has to date only been demonstrated in eukaryotes. However, the assumption that eukaryotic DNA represents a more advanced type of genetic organisation than bacterial has been suspect for some time (Reaney, *J. theor. Biol.* **48**, 243; 1974). The discovery of split genes has added a powerful buttress to the argument (for a recent, detailed discussion see Darnell *Science* **202**, 1257; 1978).

It is clear that Darnell sees DNA as retaining the genetic function in these early splicing systems. However, the points made by Darnell are consistent with a primary role for RNA both in information storage and information processing. Thus 'mosaic' RNAs produced by splicing are *strictu sensu* recombinant molecules in that they contain data drawn from different parts of the genome. But whereas classical DNA recombination requires the concerted action of a plethora of different proteins (DNA ligases, repair enzymes, denaturation proteins, exonucleases, and so on, splicing requires only two (known) functions—a cleaving and a ligating capacity. Also 'consensus' sequence targets for RNA processing enzymes are short (about six nucleotides (Catterall *et al.* *Nature* **275**, 510; 1978)) regions on single RNA strands whereas DNA recombination only occurs if two duplex DNAs of strongly matching nucleotide sequence are brought into register. Splicing thus has the virtue of economy which can in this context perhaps be equated with 'simplicity'. It seems logical therefore to suggest that RNA:RNA splicing is the primitive mode of genetic recombination.

One of the better characterised processing enzymes is RNase P. This molecule is a ribonucleoprotein containing about 80% RNA; as such it resembles an undoubtedly primitive structure—the ribosome. Stark *et al.* (*Proc. natn. Acad. Sci. U.S.A.* **75**, 3717; 1978) have raised the possibility that RNase P recognises target sequences by RNA:RNA pairing. Since known nucleotide-recognising proteins (such as the *lac* repressor) select their substrates by ex-

ploting the stereospecificity of proteins, the presence of about 350 ribonucleotides in an RNA-cleaving enzyme suggests that the processing of RNA molecules had been fixed into cell chemistry before the takeover of catalytic functions by proteins had been completed. The fact that RNase P processes the products of one of the most ancient surviving genes—that for tRNA—is consistent with this view.

The example of RNase P holds out some tantalising clues as to the origin(s) of the splicing process, conceivably at a time when effectively coded proteins had still to develop. X-ray crystallography has confirmed that the skeletons of 'working' RNAs trace out complex paths through three-dimensional space. Let us assume that RNA:RNA splicing was (and is?) mediated by RNA units which potentiate the cleavage of longer molecules at splice points ('cutter' RNA) and join shorter RNAs ('linker' RNA). (Conceivably both activities could reside in one molecule). A cutter can recognise specific bases in substrate RNA only if these are unpaired; similarly a linker can only act as a ligating agent if bases in the two separate RNAs are unpaired at or near the point(s) where the molecules are to be joined. Since the number and position of appropriately oriented, unpaired nucleotides is determined by the three-dimensional architecture of the RNA it follows that topology must have had a key role in the selection of the splice site—something which seems to survive into the present day since all (known) sequences flanking splice sites are too short *per se* to provide the needed specificity. The (at first sight) puzzling absence of strong internal Watson-Crick pairing at most known splice sites (Catterall *et al. op. cit.*) is explicable if nucleotides near the site are required to be unpaired in order to provide a recognition mechanism through base-pairing with an independent RNA.

The importance of topology can be illustrated by reference to a specific example of enzymatic interaction with an RNA of known, complex morphology—tRNA. One of the most common modifications to tRNA is methylation, carried out enzymatically using S-adenosyl methionine (SAM) as methyl donor. The function of the various methylase enzymes is to ensure that methyl groups are added at the correct site on the correct base. What is often overlooked is that it is the previous folding of the polynucleotide that determines to a large degree the availability of the bases. So by selectively shuffling base sequences so that sites to be methylated occur in loops it is possible for a low molecular weight agent such as SAM acting at random

to achieve relatively specific results. If a useful 'enzymatic' RNA sequence is found among a pool of variants this can be selectively amplified by the autocatalytic ability of duplex RNA; the reproducibility of the modifications (on which such useful enzymatic ability might depend) being ensured by the particular folding mode of transcript RNA.

What are the advantages of RNA:RNA splicing to a very early genetic system? Perhaps the most important is that the 'complexity' of polynucleotides can be increased in a modular fashion as a result of ligation between independently formed oligomers. Further, at a time when the number of effectively self-replicating RNAs was a rate-limiting factor in evolution, processing has the enormous advantage that multiple mosaics can be run off from essentially the same template; new sequences also result from the successive processings of one transcript (the production of multiple mosaics from one RNA was probably inevitable at the time when splicing enzymes had not evolved the precision needed to avoid reading-frame shifts in protein synthesis). Thus processing can maximise the number of sequences exposed to the test of selection in a way and at a rate unachievable through the slow rewriting of existing sequences by genetic noise. The adaptive advantages claimed for splicing (see Gilbert *News and Views* **271**, 501; 1978; Reaney *ICN-UCLA Symp. Persistent Viruses*, 1978) seem to apply with greater force at earlier than at later stages of evolution.

The emphasis placed on 'enzymatic' aspects of RNA implies that, in the absence of sophisticated proteins, the production of mosaics through processing may well have been easier to negotiate than the production of unit mRNAs through punctuation. Excised intronic sequences could readily be used as primers to maintain the continuing replication and transcription of template polynucleotide, thus relegating to RNA yet another role now carried out or aided by proteins.

With the takeover of genetic function by DNA, 'illegitimate' recombination may have complemented the processing mechanism. It is noteworthy that IS1 and elements of the *att* site of λ contain promotor-like sequences (Landy & Ross *Science* **197**, 1147; 1977). RNA polymerase seems to be directly involved in a *recA*-independent recombination pathway of ϕ DNA (Ikeda & Kobayashi *Proc. natn. Acad. Sci. U.S.A.* **74**, 3932; 1977). Illegitimate DNA recombination may thus preserve a memory of the primary role of RNA in early genetic processes.

Since splicing and illegitimate recombination between them provide an

enormous pool of 'experimental' polynucleotides one may ask why generalised recombination occurs across the biological spectrum. I suggest that this type of recombination developed from the proofreading function which seems to be a universal correlate of DNA synthesis. The proteins needed for generalised recombination could easily have been recruited from the molecules used in the cut and patch mechanism(s) on which proofreading depends. One of the most striking things about generalised recombination is its conservative character. No information is gained or lost and gene order is not disturbed. Significantly, branch migration and strand isomerisation show up base mismatches as transient heteroduplex lesions. Since these lesions can be recognised and repaired, generalised recombination could be regarded as an

extension of the proofreading function from which it evolved. Preferential correction to wild-type rather than mutant genotype can be achieved, at least in phage λ , by enzymatic recognition of base mismatches in non-methylated (that is, newly made) strands (Meselson & Radman cited in *Nature*, 268, 490; 1977). Thus reciprocal recombination between equal-length DNAs may, in part, be a mechanism for minimising variation, not promoting it.

According to this view the sequence of events in the evolution of genetic recombination was first, RNA:RNA splicing; second, illegitimate recombination and third generalised recombination. Legitimate recombination now acts as a brake on change, continuing evolution being nourished by the earlier mechanisms.

□

Short period fluctuations in the Earth's magnetic field

from Ken Games

Up to about 10 years ago, it was generally believed that the magnitude of the Earth's magnetic field varied with periods of approximately 8,000 years on a world wide scale. Thus in 1968 Cox (*Science* 163, 237; 1968) produced a graph of variation in the geomagnetic dipole moment which was obtained by averaging data over 500 year intervals (Fig. 1). In recent years, however, researchers are finding local changes in the magnitude of the magnetic field which have periods of as little as 200 years and which show a change in the total field strength of up to 100%. The questions which now have to be answered are: how confident are we in the magnetic measurements used to determine these changes, and, if these changes are real, what is causing them?

The commonest material used in field magnitude determination is pottery. The main technique used to 'unlock' the magnetic record held in the pottery is that developed by Thellier and Thellier (*Ann. Geophys.* 15, 285; 1959), although many modifications to this technique have since been made. The basic Thellier and Thellier method involves heating a sample to, say, 100 °C in zero magnetic field and allowing it to cool to room temperature. The sample is then reheated to 100 °C in a known laboratory field B_{lab} and again cooled. The decrease in magnetic moment m_1 after the first heating represents the magnetisation acquired by the sample in the temperature interval

R.T. - 100 °C when the sample was first fired in the ancient magnetic field B_{anc} , and the increase in magnetic moment m_2 after the second heating represents the magnetisation acquired in the laboratory field. Thus the simple equation

$$\frac{m_1}{m_2} = \frac{B_{anc}}{B_{lab}}$$

enables us to determine B_{anc} . This process is then repeated at steps of increasing temperatures such as 200, 300, 400 and 500 °C. If the value of B_{anc} obtained from each step is the same then the result is assumed to be a reliable one. Several possible sources of error in this method have been known and taken into account for a long time. For example, the laboratory heating can cause changes in the magnetic minerals of the sample. This tends to happen at high temperatures, and many of the modifications of the method have attempted to monitor such changes.

Recently, however, two serious possible sources of error have been discovered. The first source was reported by Fox, Aitken and Gunn (*Geophys. J. Roy. Astron. Soc.* 53, 153; 1978) when they discovered a shape anisotropy effect. Thus it was found that the thermoremanent magnetisation (TRM) acquired by a disk-shaped sample (25 mm in diameter and 6 mm thick) along the plane of the disk was about 20% greater than that acquired perpendicular to the plane. This effect seems to be similar to the self-demagnetising effect exhibited by kilns as shown by

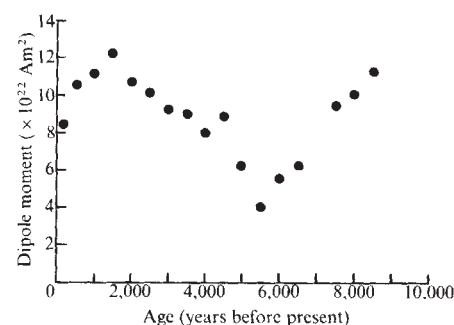


Fig. 1 Variations in geomagnetic dipole moment. Changes during the past 130 yr, as determined from observational measurements, are shown by the slanting bar at the left. Other values were determined palaeomagnetically (Cox *op. cit.*).

Aitken and Hawley (*Archaeometry* 13, 83; 1971).

The second source, reported by Rogers, Fox and Aitken (on page 644 of this issue of *Nature*) is another anisotropic problem, this time shown to exist on cylindrical samples 3 mm x 3 mm, which should therefore have no anisotropy due to shape. However, it was found that for samples whose cylindrical axis was perpendicular to the plane of the pottery, the TRM along the axis was up to 40% less than the TRM perpendicular to the axis. This is thought to be due to a preferential alignment of magnetic grains within the fabric of the pottery. One would intuitively expect that this effect would be greater for wheel-made pottery than for hand-moulded pottery, but whether this is so or not, it obviously presents a problem in interpreting archaeomagnitudes which have been reported before this discovery. What is certain is that in future care should be taken to give the TRM in the same direction as the normal remanent magnetisation (NRM) was originally acquired, or at least to correct for the anisotropy effect. Even so, there is a further problem yet to be investigated. This is the fact that when a sample first acquired its NRM it was part of a larger mass of ceramic, and no one really knows just what difference this makes compared with the acquisition of the TRM after it has been broken away from the larger mass.

One author who has made a correction for the fabric anisotropy is Walton (on page 643 of this issue). His results show a rapid rate of change in the magnitude of the magnetic field in Greece between 2000 BC and AD 400. As stated earlier, these are the sort of results now being found by several workers, and it becomes apparent if the diagram in Fig. 1 is compared with that of Walton, that by averaging over 500-yr intervals Cox has automatically lost the resolution which, given adequate precision of dating, could show the rapid short-period fluctuations now

being seen. It is also clear that it becomes of vital importance to use material which is very accurately and reliably dated—preferably to within ± 30 yr. Where such dating controls exist, the magnetic results usually show the rapid short period fluctuations.

The major problem still to be answered, however, is what is causing these fluctuations, which can result in a change in magnitude of the magnetic field of up to a factor of 2 in a couple of hundred years. It is difficult to see how the main dipole field can change at such a rate, but before this can be discounted as a possibility we would need to have archaeomagnitudes for the same time period from many different locations, so that an accurate picture of the world-wide rate of change could be produced. The other obvious source of the fluctuations is the non-dipole field. If such non-dipole features did exist at various localities, then as they are so large they must be observable over large areas of the Earth's surface.

Hence a study of archaeomagnitudes from areas separated by, say, 1,000 or 2,000 km should enable us to determine how these non-dipole features grew and decayed, and whether or not they showed the Westward drift which is a feature of the present-day non-dipole field.

As far as the future is concerned, detailed curves of the magnitude of the Earth's magnetic field must be produced for many different areas if we are to understand the reason for the rapid fluctuations now being observed. The material used for this work must be well dated, and as Walton explains, the possibility of then using the curve produced for a given location as a dating tool would be of great help to archaeologists. However, as with all methods of archaeological dating, we must proceed slowly to ensure that the curves we use are as accurate as possible, and that as many sources of error as possible, such as the fabric anisotropy just discovered, are eliminated.

The juxtaglomerular complex: its possible control of multiple nephron functions

from Arthur C. Guyton and John E. Hall

THE discovery of a new secretory cell in the juxtaglomerular complex of the renal nephron is announced in this issue of *Nature* (page 655) by Ryan, Coghlan, and Scoggins. This cell, the granulated peripolar epithelial cell, contains large numbers of granules that are obviously secretory in nature, and it is located in the angle between the glomerular tuft and the outside wall of Bowman's capsule, at a place where its secretion passes directly into the glomerular filtrate as it is first formed at the glomerulus. The authors make the obvious observation that "it is almost inescapable that they [the cells] participate in juxtaglomerular complex [control] activity". But what is the function of these secretory cells?

The juxtaglomerular complex is an anatomical consortium of structures lying at the pole of the glomerulus. It consists of a short portion of the afferent arteriole immediately before it enters the glomerulus and similarly a short portion of the exiting efferent arteriole. And, very important, it also includes a small segment of the distal tubule that crosses the glomerular pole in the angle between the afferent and efferent arterioles and abuts against both these arterioles. This is an invariable structure of each nephron in all higher orders of animals. Obviously, this location of the complex is most propitious for it to act as a controller of nephron function, so much so that essentially every anatomist who has made contributions to its structure has

referred to its possible control functions. These include Ruyter (*Ztschr. f. Zellforsch. u. mikr. Anat.* **2**, 242; 1925) who described its general structure, Goormaghtigh (*Comp. Rend. Soc. Biol., Paris* **124**, 293; 1937) who emphasised the importance of 'juxtaglomerular' myoepithelial cells in the arterial walls containing large numbers of secretory granules, McManus (*Q. Jl Micr. Sci.* **88**, 39; 1947) and Schloss (*Acta. Anat., Basel* **1**, 365; 1946) who further defined the interrelationships between the distal tubule and the vascular pole, Hartroft (*Circ. Res.* **12**, 525; 1963) who helped to determine that the secretory granules of the juxtaglomerular cells contain large quantities of renin or prerenin, and many others. In fact Schloss gave the juxtaglomerular complex the name '*der Regulationsapparat*'.

Yet, how much do we know about control of nephron function by the juxtaglomerular complex? The answer is that there has been very little proof of any control function, but on the other hand, large amounts of circumstantial evidence favour several different control functions. In 1964 (Guyton *et al. Circ. Res. (Suppl. 1)* **14**, 1; 1964), we summarised some of this circumstantial evidence and also presented a mathematical model of the manner in which feedback control signals from the distal tubule could

'autoregulate' both nephron blood flow and nephron glomerular filtration rate. Leyssac also suggested a similar mechanism (*Acta physiol. Scand.* **58**, 236; 1963). Since then, many renal physiologists have presented evidence for and against such a method for autoregulation of renal blood flow and glomerular filtration rate. After a long period in which it was disbelieved by most renal physiologists, sentiment seems to be shifting toward acceptance of such a mechanism, so much so that a new term has been coined to describe it, 'tubuloglomerular feedback'. However, there is still much disagreement over what chemical constituent or what physical factor in the distal tubular fluid is sensed to provide the feedback control of nephron blood flow and glomerular filtration.

Nephron function is also controlled by the renin-angiotensin system. At first it was thought that the renin-angiotensin system caused constriction of the afferent arterioles in response to excess sodium or chloride ions in the distal tubules (Thurau & Schnermann *Klin. Wochschr.* **43**, 410; 1965), in this way providing feedback control of the rate of flow of the sodium and chloride ions through the tubular system. However, with the advent of drugs that can block the renin-angiotensin system, we and others have been able to show that autoregulation of renal blood flow becomes better than ever when the renin-angiotensin system is blocked (Hall *et al. Am. J. Physiol.* **232**, F215; 1977). On the other hand, autoregulation of glomerular filtration is better when the renin-angiotensin system is functional. These studies have also allowed us to separate tubuloglomerular feedback into two separate components, one of which controls afferent arteriolar constriction and does not involve the renin system, yet has a strong influence on autoregulation of renal blood flow, but only a weak influence on the autoregulation of glomerular filtration. The second feedback mechanism does involve the renin system which controls the degree of vasoconstriction of the efferent arterioles. That is, when glomerular filtration is too low, feedback from the distal tubule to the juxtaglomerular cells causes renin secretion, formation of angiotensin, constriction of the efferent arteriole, elevation of glomerular pressure, and return of the glomerular filtration rate towards normal.

Thus, the efferent vasoconstrictor mechanism provides an additional quantitative dimension to glomerular filtration autoregulation, adding to the effect also provided by the afferent arteriolar mechanism.

So what control does the secretion from the newly defined peripolar cells exert. As this secretion enters directly

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into the glomerular filtrate, one would suspect control of some aspect of tubular reabsorption. The obvious first question is its possible role in controlling sodium reabsorption, a subject that is still lost in a haze. But we can all think of many other nephron control functions that we would like to see it perform.

Thus, the importance of the juxta-glomerular complex grows, not merely for control of a single function of the nephron but perhaps and probably as a controller of multiple functions. □

Trouble with time travel

from Paul Davies

SPECULATION about time travel has long been a source of inspiration to science fiction writers and philosophers, but anathema to physicists. Of course, time travel in a limited sense is known to be an observable fact. Ever since the inception of special relativity, with its celebrated time dilation and 'twins' effects, physicists have recognised that a sufficiently powerful rocket would enable an astronaut to travel into the future. By approaching close to the speed of light, a trip which for the astronaut occupies, say, one year, could return him to Earth to find that fifty years had elapsed there. Indeed, the disparity can theoretically be made as great as one pleases by approaching arbitrarily close to the speed of light. The phenomenon is not just a conjecture. Time-travelling subatomic particles are routinely monitored in the laboratory, and the minute, but significant, time dislocations in jet aircraft, have been successfully measured. However, the trip is one way only: there is no going back into the past.

The more entertaining possibility of visiting one's own past is the one that causes physicists headaches, for it plays havoc with causality. Suppose a time traveller kills his parents before his birth? Even signalling the past can cause horrendous paradoxes. Consider the machine that destroys itself at two o'clock if it receives a message from itself at one o'clock, sent backwards in time from three o'clock. If it blows up at two, no message goes out at three, so no explosion is ordered.

It could be argued that a mass of contradictions can be avoided if one only allows a limited range of causally self-consistent loops, thus ruling out 'autocidal' machines and so on *ad hoc*. But there is no doubt that the world

would be a safer place if some law of physics could be found that forbids travel into the past. The trouble is that, not only do we not know of such a law, we actually do know of some circumstances where our existing theory predicts just such a possibility. The general theory of relativity, which allows space-time to be bent and distorted, describes situations in which world lines (tracks of matter through space-time) are bent right back round and can even 'close up' on themselves, that is, join up with their own past. Thus, a particle can find that its past is also its future—a collapse of causality that few are prepared to take seriously.

One situation in which general relativity seems to predict (if that is the correct word) causality violation is in the vicinity of rapidly rotating massive objects, a celebrated case being the inside of a spinning black hole. Another possibility occurs near a rotating massive cylinder (a more promising case for would-be temponauts). The experts have long regarded mathematical demonstrations of this sort as idealisations, and supposed that in reality some kind of instability would set in to prevent the causality violation occurring. There is still a lack of any comprehensive theorems which will demonstrate that general relativity alone saves itself from its own causality-violating solutions, but in 1976, Frank Tipler of the University of California proved that time machines tend to go hand in hand with space-time singularities, also considered to be extremely unpalatable by physicists. So perhaps ridding the theory of singularities would also save causality.

A different possibility has recently been explored by an undergraduate student from Bristol University, now a postgraduate at King's College, London. Writing in a recent issue of the *Journal of Physics* (Charlton, *J. Phys.* **11**, 2207; 1978) he first investigates the topology of the two-dimensional surface which surrounds the region of spacetime in which particles possess closed timelike world lines, proving it is always a torus. He then goes on to discuss the behaviour of photons close to the time machine region. The result which he finds is that the angular momentum carried by the photon rises without limit as the conditions are approached for the existence of causality violation. In physical terms this means that any loss of radiation from the region will transport vast quantities of angular momentum away from the rotating body. The reaction back on the body itself will cause it to spin down, and hence retreat from the threat of causality violation. Thus, there seems to be an inbuilt mechanism to prevent real objects from rotating fast enough to allow time travel.

Just how general Charlton's mechanism will turn out to be is not yet clear, but it does go some way to confirm what has long been suspected—that travel into the past is purely a fiction. □

Lunar origin and palaeotides

from David W. Hughes

BEFORE the Apollo missions to the Moon there were three seriously held hypotheses for its origin. The earliest is just over a hundred years old. George H. Darwin suggested in 1878 that in early times part of the Earth's crust broke away to produce the Moon, leaving the Pacific Ocean as a scar. This occurred when the Earth had already differentiated into a core and a mantle and was rotating with a period of ~ 4 h. This low spin period caused it to become oblate and unstable. More recent additions to this hypothesis suggest that Mars and a myriad of smaller bodies broke away at the same time.

The second hypothesis proposes that the Moon was formed as an independent Solar System planet, further away from the Sun than Earth, and was captured by Earth about 4×10^9 yr ago.

Ring accretion is the basis of the third hypothesis. Earth once had a ring system that may have resembled that of Saturn, the planetesimals within the rings either being derived from Earth by silicate precipitation in a massive high temperature atmosphere or being the remnants of the original condensing planetesimal group that formed the Earth. This ring coagulated after it had receded beyond the Roche limit.

Following N. M. Short (*Planetary Geology*, Prentice Hall, 1975) we can list some of the important lunar characteristics that the origin hypotheses have to explain. The Moon is very close to being a homogeneous sphere; it has a lower density than Earth, indicating that it has lost iron; there is no water and fewer volatile and siderophile elements than both the Earth and chondritic meteorites, the Moon seems to be enriched in refractory elements, and obviously has a none too primitive composition; it was formed about 4.6×10^9 yr ago, the highland rocks being about 4.3×10^9 to 4.1×10^9 yr old, the mare basalts being about 3.7×10^9 yr old. It seems that the Moon was formed essentially at the same time as Earth and that the majority of the surface evolution took place in the

first 10^9 years; also there are indications that the Moon was once much closer to the Earth than it is at present.

Unfortunately none of the pre- or post-Apollo discoveries compels the unanimous acceptance or rejection of any of the three hypotheses listed above. The authors of these hypotheses, to a man, have found something in the data that apparently supports their point of view. However, A. J. Anderson (University of Uppsala, Sweden) is convinced that a detailed study of the figure of the Moon and the tidal forces acting on it in the past (the so-called palaeotides) indicate that the Moon could not have been formed in orbit about the Earth. This would automatically rule out the ring accretion and fission hypotheses, leaving only capture. Anderson presents his case in a recent edition of *The Moon and The Planets* (19, 409; 1978).

The non-hydrostatic figure of the Moon arose from a distortion introduced by tidal forces some time in the past, a distortion which has been retained by the finite strength of the lunar interior. The gravity field can be charted using Doppler tracking techniques from the Orbiter spacecrafts and the Apollo command service modules that have circled the Moon. This technique led to the discovery of mascons, positive gravity anomalies that existed over certain circular lunar maria basins. These are probably thin concentrations of excess lava that have existed on the Moon for about 3.6×10^9 yr. Isostatic global readjustment has not occurred simply because the viscosity of the lunar lithosphere is of the order of 10^{27} – 10^{28} poise, about five orders of magnitude greater than the Earth's.

When the maria were formed by the filling of basins with low-viscosity molten lava the resulting maria surfaces would coincide with the gravitational equipotentials. A careful analysis of the present-day figure of the maria surfaces gives the remnant static tidal gravity field acting at the time of solidification, and consequently the Earth–Moon distance at that time.

Anderson finds that the older lunar highlands have a smaller tidal ellipticity than the younger circular mascon maria. This is completely contrary to the supposition that the Moon was closer to the Earth when the highlands were formed than it was when the maria were formed. But we know that tidal friction does cause the Moon to recede slowly, in fact the time the Moon spends at a distance R from the Earth is proportional to R^n where n lies between 3.5 and 4.5. Anderson therefore concludes that the Moon was formed away from the Earth and was captured about 3.7×10^9 yr ago just before the time of mascon formation.

There is a possibility that the lava flow which produced the mascons was actually caused by the capture. In producing this static tide solution Anderson has to assume spin-orbital coupling. This leads to the further conclusions that the mascon maria flows occurred when the Moon was between 8 and 10 Earth radii away from Earth and that before this the Moon was locked in a configuration with the Earth–Moon line about 15 degrees to the east of its present value. A remnant bulge on the lunar far side, revealed by the Apollo 16 laser altimeter, confirms the fact that just such a re-orientation has occurred.

So we are left with the lunar capture hypothesis, a hypothesis which un-

fortunately completely dissociates the origin of the Moon from the origin of the Earth and relegates the cause of their present companionship to a chance encounter during the dawn of the Solar System. If we look at other planetary satellites we can draw comfort from the fact that of the seven lunar-sized satellites, two of them (Moon and Triton) do not have orbital planes which coincide with the equatorial planes of the present planets. Considering all the satellites, about 38% are in non-equatorial orbits, in fact 19% are retrograde. It seems that satellite capture was a fairly common occurrence during the formation of the Solar System.

□

Pathogenesis of animal trypanosomiasis

from F. E. G. Cox

ANIMAL trypanosomiasis, particularly in cattle, is a major constraint on meat production in Africa. Whether or not the presence of trypanosomiasis, which effectively prevents large scale cattle ranching throughout the tsetse fly belt, is a factor in the preservation of African soil against erosion (Ormerod *Science* **191**, 815; 1976) can be debated but there is little doubt that animals suffering from this disease are not productive and therefore constitute a considerable economic drain on the countries of Africa. Much is known about the symptoms of trypanosomiasis but little is understood of the pathogenesis of the disease and a recent meeting* brought together a number of scientists working on this and related problems.

The most important species of trypanosome causing animal trypanosomiasis are *Trypanosoma vivax*, *T. congolense* and *T. brucei* which infect cattle, sheep and goats as well as wild and laboratory animals. The pathogenic effects vary from parasite to parasite, host to host and even individual to individual. This is partly because the trypanosomes occupy different sites in the body, *T. congolense* and *T. vivax* are normally confined to the blood while *T. brucei* is found in both blood and tissues, and partly because pathogenic changes may be caused by the trypanosomes themselves or by the

hosts' efforts to rid themselves of their infections. All three species undergo antigenic variation so in most animals there is little or no protective immunity while ineffective immune responses bring about a number of deleterious immunopathological changes.

M. Murray (ILRAD, Nairobi) suggests that the pathogenic changes during trypanosomiasis in cattle proceed in three phases; anaemia with high parasitaemia and enlarged spleen, anaemia with transient parasitaemia and variable spleen size and, in those animals that recover, anaemia without parasitaemia and the spleen returning to normal. However, dramatic changes occur within a matter of hours and many cattle die without showing major signs of disease (G. Losos, Veterinary Research Department, Muguga, Kenya). The inadequacy of the immune response is indicated by the fact that animals challenged a year after recovery usually go through the whole cycle again. During infection, irreversible lesions occur and complement levels, for example, never return to normal (H. Tabel, University of Saskatchewan).

There are probably several causes of the characteristic anaemia of trypanosomiasis. There is little doubt that a haemolytic factor is involved and one possible candidate is a phospholipase associated with dying or dead trypanosomes (I. Tizard *et al.* University of Guelph). This is the first defined toxic product of a trypanosome to be implicated in the pathogenesis of trypanosomiasis and the long search for such substances, begun in the time of Landsteiner, is obviously well worth continuing. There is also evidence that

*A Conference on Recent Advances in the Knowledge of Pathogenicity of Trypanosomes sponsored by the Canadian International Development Agency (CIDA) and International Development Research Centre (IDRC) and the International Laboratory for Research on Animal Diseases (ILRAD) was hosted by the Veterinary Research Department of the Kenya Agricultural Research Institute, Muguga and was held in Nairobi, Kenya, on 20–23 November, 1978.

immunological responses initiated by the trypanosomes themselves are involved in red cell lysis (Tabel).

Following the initial infection, pathogenic changes occur so rapidly that it is almost impossible to unravel those that result directly from the presence of the parasites from those resulting from the hosts' attempts to compensate for damage caused by anaemia and immunological responses to parasite and possibly host antigens. Nevertheless, the disease has many of the characteristics of an inflammatory one and P. F. L. Boreham (Imperial College, London) postulates that antibody-antigen complexes initiate a series of reactions leading to the activation of kallikrein and eventually increased capillary permeability on one hand and disseminated intravascular coagulation on the other. These effects, and the anaemia, are only part of the whole pattern of pathogenesis and virtually every kind of cell (M. G. Maxie & V. E. O. Valli, University of Guelph), tissue (W. I. Morrison & M. Murray, ILRAD, Nairobi) and organ (Valli) may be involved. The dynamics of the various changes can now be determined very accurately using a number of tracer and radioactive labelling techniques (J. D. Dargie, FAO/IAEA, Vienna) and these techniques will become increasingly useful in the next stage of unravelling the various intertwined reactions.

No conference on trypanosomiasis could be complete without some consideration of the ways in which meat-producing animals can be kept in regions of endemic trypanosomiasis. The possibility of using cattle tolerant to trypanosomiasis is not an immediate one as carefully controlled studies have now shown that the reputed trypanotolerant breeds like the N'dama do suffer considerably from trypanosomiasis although not as much as Zebu (Murray, Dargie) but much more quantitative work is required on this subject. On the other hand, young cattle do not seem to suffer so severely from trypanosomiasis as do adults (B. T. Welde, Walter Reed Institute Washington) and eland that can be bred in captivity do seem to recover from infection (R. Olubayo, Veterinary Research Laboratory, Kabete, Kenya). Perhaps we should acknowledge the supremacy of the tsetse fly at least temporarily and turn our attention to less popular breeds of cattle and alternative meat-producing animals which will eventually be able to live in Africa together with the tsetse fly and the trypanosomes but in the absence of clinical trypanosomiasis. □

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Pacific quiet zone contracted

from Peter J. Smith

LARGE-amplitude linear magnetic anomalies have now been used to interpret vast areas of the Earth's ocean floor in terms of plate tectonic processes. But there are still gaps; there are still difficulties in some areas, not least in the western Pacific. The late Mesozoic pattern of magnetic lineations in this region was first unravelled by Larson and Chace (*Geol. Soc. Am. Bull.*, **83**, 3627; 1972) who traced seafloor spreading back to the early Cretaceous and later to 153 million years (see, for example, Larson & Hilde *J. geophys. Res.*, **80**, 2586; 1975). But beyond that, in both temporal and geographical senses, there is a large zone of low-amplitude (<100 γ) anomalies, known as the Pacific Jurassic quiet zone (PJQZ), surrounding the Mariana Basin.

Whatever the cause of the PJQZ, its existence has impeded the resolution of the area's tectonic history and may have led to unnecessarily forced interpretations. For example, Hilde *et al.* (*Tectonophys.*, **38**, 145; 1977) suggested that the Mariana Basin may be underlain by Cretaceous crust formed during the better-documented Cretaceous magnetically quiet period—a proposal which implies, of course, that the basin originated during a time of intraplate spreading about 100 million years ago. In other words, by this interpretation, the magnetic anomalies in the western Pacific get older and older until they reach the PJQZ boundary at 153 million years, beyond which the lithosphere is younger.

This is possible, but unlikely. Moreover, as Cande *et al.* now suggest (*Earth planet. Sci. Lett.*, **41**, 434; 1978), such contorted theorising is unnecessary. Cande and his colleagues show (from existing data) that in the PJQZ, immediately beyond the 153 million year line, the magnetic anomalies are apparently no different in character and lineation, but differ only in amplitude, from those on the younger side of the line. It is necessary to say 'apparently' here because it is just possible that the older alternating positive and negative anomalies are not the result of field reversal at all but of field magnitude fluctuations within a single polarity; and certainly the observed anomalies may be modelled successfully on either assumption. But just as there is no doubt that the anomalies younger than 153 million years are produced by a combination of seafloor spreading and geomagnetic field reversal, there is little reason to suspect that the older

anomalies are not produced likewise. Cande *et al.* have thus been able to extend the Mesozoic polarity-time scale, temporally about 5 million years beyond the previous limit of 153 million years and geographically closer in towards the Mariana Basin from three different directions.

On this basis the field reversal rate during those 5 million years within the PJQZ was calculated to be once about every 100,000 years. But if the 153 million year boundary has no significance in terms of field reversal, seafloor spreading and the shape of the resultant magnetic anomalies, why is there a PJQZ at all? The first thing to be said about this is that the PJQZ boundary is not as sharp as it is sometimes imagined; there is no sudden change in anomaly amplitude at 153 million years. In fact, the amplitude begins to decrease somewhere around 140 million years and continues to do so fairly smoothly up to 155–160 million years. Or to rephrase this in forward time terms, from 160 to 140 million years ago the anomaly amplitude in the western Pacific increased fairly smoothly, actually by a factor of about four.

This increase in amplitude could have been due to a real increase in the magnitude of the geomagnetic dipole moment. Alternatively, it could have been the result of a gradual decrease in the frequency of (undetected) short polarity events, the presence of which would give an apparent reduction in the amplitudes of the observed anomalies. On the basis of the small-scale magnetic detail observed in the profiles from the faster-spreading sections of the western Pacific, Cande and his colleagues conclude that the first of these explanations is the most likely. Either way, the field of uncertainty in the western Pacific has been reduced, and in such a way as to suggest that further reduction may soon be possible. □

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A hundred years ago

At the last meeting of the French Geographical Society a letter was read from the Abbé Desgodins, dated Yerkalo, August 27, 1878, in which he states that, contrary to the common assertion which represents the sheep as the beast of burden most used in Thibet, this function belongs in preference to the yak (*Bos grussiens*); the mule, ass, and horse are also made use of.

From *Nature* **19**, 20 February, 375; 1879.

review article

Laser isotope separation

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It is about 10 years since new methods of isotope separation by means of laser radiation were first proposed. In the past five years a lot of effort has been put into elaborating and realising these techniques. Now we are at the threshold of demonstrating the first pilot set-ups of laser isotope separation (LIS). In this review I will try to outline the basic ideas, methods and key experiments.

THE successful elaboration of LIS methods at the beginning of the 1970s became possible only because of the development of a variety of tunable lasers covering a wide spectral range from about 2,000 Å to 20 μm. Moreover, LIS methods pose stringent requirements for the parameters of tunable lasers and thus stimulate additional research into the development of highly efficient tunable lasers. (For more detailed reviews see refs 1–5.)

Ideas and various LIS methods

All LIS methods are based on a series of consecutive processes which start with the selective photo-excitation of an atom or a molecule of the chosen isotopic composition. Isotopically selective photoexcitation is possible because of the difference of frequencies and structure of spectral absorption lines of atoms and molecules of different isotopic composition^{6,7}. Isotopic effects appear in optical atomic spectra due to the difference of masses, volumes and nuclear spins of different isotopes^{8,9}. The difference in isotope mass which affects the frequency of vibrations and rotations of a molecule is essential for molecular spectra^{10–12}. Spectral lines in a gas of low pressure (less than a few torr) are very narrow, so that a small isotopic shift is sufficient for selective excitation of the chosen isotope. However, there are some important exceptions (atomic spectra of alkaline and alkaline-earth elements, spectra of polyatomic molecules with heavy isotopes at room temperature) for which special methods of narrowing of spectral lines (atomic beams¹³) and of simplification of complex molecular spectra (cooling of molecules in a gas or in a supersonic jet^{14–16}) are applied. Broadened spectral lines of molecules in a gas at a pressure of more than a few torr and in a condensed medium are not appropriate with rare exceptions (low temperature matrices¹⁷) for isotopically selective excitation.

The excitation of an atom (molecule) changes some of its physical and chemical properties (Fig. 1) noticeably. First, the excited atom (molecule) usually has a higher chemical reactivity than the unexcited one, as the acquired internal energy increases the probability of overcoming the energy barrier of a chemical reaction. The photochemical approach to isotope separation is based on the ability of selectively photoexcited atoms (molecules) to react chemically with a suitable partner at a rate considerably exceeding the rate of chemical reaction of unexcited atoms (molecules) of another isotopic composition. This approach is well known and was successfully used in the photo-excitation of electronic states of some atoms and molecules before lasers were invented^{18–24}.

Second, the excitation of an atom markedly decreases its ionisation energy and hence selectively excited atoms can be ionised by additional radiation of a suitable wavelength, leaving unexcited atoms of different isotopic composition untouched. This idea forms the basis of the atomic photoionisation approach to LIS.^{25–27}

Third, the excitation of a molecule reduces its dissociation energy quite significantly and hence selectively excited molecules can be dissociated much more easily than unexcited molecules of another isotopic species. This mechanism is the basis of the first photodissociation methods of LIS developed^{27–29}.

These three main ideas are the basis of the fundamental actively developed trends (photochemistry, photoionisation and photodissociation) in LIS considered here. Several other less universal, but also interesting, methods of LIS use photopredissociation^{30,31} and photoisomerisation³² of some molecules, and photodeflection of the atomic trajectory at photon absorption due to the recoil effect³³; other methods are also proposed and demonstrated.

Photoionisation methods

Selective photoionisation of atoms is the most universal photo-physical method for selective separation of substances, particularly isotopes, at the atomic level. A feature common to all

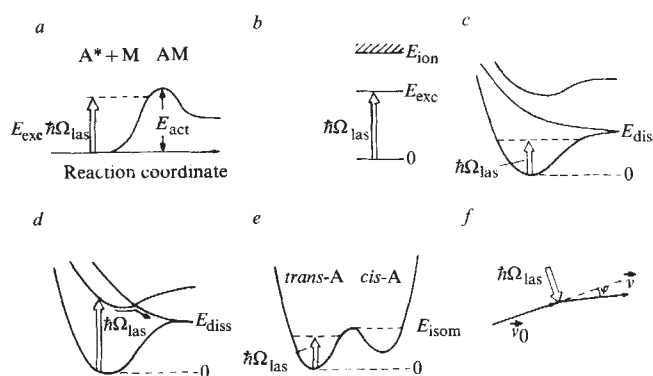


Fig. 1 Atomic and molecular properties which vary during laser excitation: (a) enhancement of reactivity; (b) reduction in ionisation energy; (c) reduction in dissociation energy; (d) photopredissociation; (e) photoisomerisation; (f) change in mechanical trajectory.

schemes for selective ionisation, is the sequence of the two steps: (1) isotopically selective excitation and (2) ionisation of the excited atoms. Figure 2 shows the main schemes of selective atomic ionisation of special interest for LIS. The two-step photoionisation scheme²³⁻²⁷ is the simplest. The three-step scheme may be useful for ionisation of atoms with a high ionisation potential using visible lasers. The photoionisation cross-section can be increased by tuning the frequency of the last-step radiation to that of a transition to an autoionisation state: either a spontaneous³⁴ or an electric field-induced³⁵ state. Finally, high Rydberg states can be ionised by IR radiation³⁶ or pulsed electric fields³⁵. All these schemes have already been used for isotopically selective ionisation. Their advantages and disadvantages are not discussed here but detailed information is given in ref. 37.

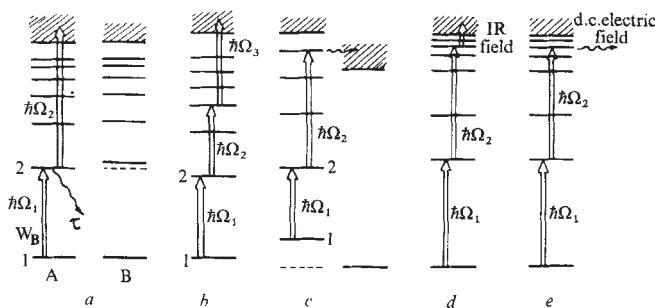


Fig. 2 Mechanisms of isotopically selective step-wise photoionisations of atoms by laser radiation; *a*, two-step photoionisation; *b*, three-step photoionisation; *c*, stepwise photoionisation through an autoionisation state; *d*, selective excitation of a Rydberg state and its photoionisation by IR laser radiation; *e*, selective excitation of a Rydberg state and its ionisation by electric field.

A great deal of attention has been paid to uranium isotope separation (some research programmes are described in refs 38-42). At low density of uranium vapour in an atomic beam (10^{10} cm^{-3}), a 70% isotope enrichment of ^{235}U uranium ions has been obtained as a result of selective two- or three-step ionisation of ^{235}U atoms by dye laser radiation. As the abundance of ^{235}U in natural uranium is only about 0.7%, such an enrichment corresponds to an isotope separation factor of about 100. The operating conditions of the LIS set-up at such low vapour density in the atomic beam are far from optimal and in order to obtain the necessary production capacity and high efficiency of the applied laser radiation the size of the equipment should be very large. Similar experiments at higher density of uranium vapour ($10^{13} \text{ atoms cm}^{-3}$) have yielded 6% enriched ^{235}U (ref. 42). This indicates that this process is well adapted to produce light-water reactor fuel but less suitable for highly enriched uranium. The considerable technical problems of such a method of uranium LIS, for instance, the high chemical reactivity of uranium vapour at the high temperatures necessary to produce the required density should also be kept in mind. Therefore there are some problems in industrialising the atomic ionisation process of uranium LIS. But the suggestion that such a process will lead to the cheap production of highly enriched uranium in moderate-size laboratories, is somewhat exaggerated⁴³.

Besides the uranium isotope separation, which demands rather different economic technological conditions, laboratory experiments are being carried out on the selective ionisation of other isotopes, which are potentially useful in much smaller quantities, such as Ca (ref. 13), Rb (ref. 44), rare-earth⁴⁵, and probably transuranium elements. Effective ionisation of excited atoms at a moderate average intensity of the ionising laser radiation, on laboratory LIS set-ups presents a major problem in these cases. The ionisation schemes given in Fig. 2*a,b* cannot be practically applied here, and so greater attention must be paid to the ionisation scheme of highly excited atoms shown in Fig. 2*d,c*. The ionisation of high-lying states by an electrical field pulse^{35,44,46} is the most simple and universally applied method.

This method of stepwise ionisation has been reported in detail elsewhere⁴⁶⁻⁵⁰ for various atoms.

The discovery of a long-lived ($\sim 1 \text{ ns}$) autoionisation state in the spectrum of Gd atoms⁵¹ with its large transition cross-section of 10^{-15} cm^2 shows that such a scheme can sometimes compete with the scheme which uses the electrical pulse for ionisation of a highly excited atom.

The method of multi-step selective ionisation of atoms through the high-lying sharp quantum states in the vicinity (below or sometimes above) of the ionisation limit is quite suitable for LIS, at least on a laboratory scale.

Photodissociation methods

Various schemes of isotopically selective photodissociation of molecules through the excited and/or the ground electronic state are shown in Fig. 3. These were first proposed, experimentally realised and used for isotope separation at the Institute of Spectroscopy of the Academy of Sciences of the USSR. They are now widely used both for LIS and for potential applications in photochemistry, phototechnology of materials, and most recently, in photobiology.

All these schemes are based on primary isotopically selective excitation of vibrational states of molecules by IR laser radiation as long as the vibrational spectra have considerable isotopic shifts (especially for light elements). In the simplest case (Fig. 3*a*) the molecules excited to the first vibrational level can be dissociated by irradiation of an appropriate wavelength^{28,29} following the excitation of the unstable electronic state. The first successful experiments on isotope separation using two-step (IR + UV) molecular photodissociation were carried out on molecules of $^{14}\text{NH}_3$ and $^{15}\text{NH}_3$ (refs 29, 52) and the corresponding experiments on molecules of $^{10}\text{BCl}_3$ and $^{11}\text{BCl}_3$ were carried out⁵³ later.

The disadvantage of such a method is the comparatively small redshift of the UV photodissociation absorption band after excitation of a molecule to the first vibrational level. This limits the isotopic selectivity of IR + UV dissociation. This disadvantage disappears when exciting higher vibrational levels due to the multi-step or multi-photon process (Fig. 3*b*). This has recently been shown in experiments⁵⁴ with $^{12}\text{CF}_3\text{I}$ and $^{13}\text{CF}_3\text{I}$ molecules using an IR CO_2 laser and UV excimer laser, where the dissociation selectivity has been found to be >100 .

An impressive and productive discovery was the isotopically selective photodissociation of polyatomic molecules BCl_3 ⁵⁵, SF_6 ⁵⁶ by intense IR CO_2 laser radiation. The discovery of this effect was preceded by studies⁵⁷⁻⁶¹ of the interaction of intense IR CO_2 laser pulses with molecular gases absorbing in the region of $10 \mu\text{m}$. It was found that under relatively intense IR radiation during a short time (10^{-7} – 10^{-9} s), a polyatomic molecule could pass through the vibrational levels reaching even the dissociation limit (Fig. 3*c*), without colliding with other molecules. The whole process of multiple absorption of IR photons begins

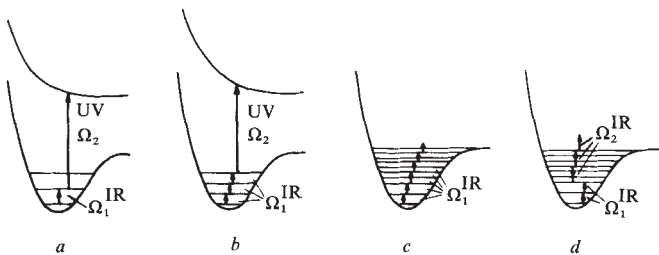


Fig. 3 Schemes of isotopically selective multi-step photodissociation of molecules by laser radiation through an excited electronic state (*a, b*) and the electronic ground state (*c, d*): *a*, two-step IR + UV photodissociation; *b*, multi-selective excitation of high vibrational levels and their photodissociation by UV radiation; *c*, multiple photon selective excitation and dissociation by a single frequency intense IR field; *d*, multiple photon selective excitation of vibrational levels by resonant IR field and their multi-photon dissociation by non-resonant intense IR field.

with a low vibrational transition where there is usually a marked isotopic shift (at least for isotopes of light and moderate masses). By choosing an appropriate IR radiation frequency the dissociation of some isotopic molecules can be achieved. The case of isotopic SF_6 molecules is shown in Fig. 4. When the frequency of powerful pulsed CO_2 laser radiation with pulse energy of several J cm^{-2} is tuned to the absorption frequency of the chosen isotopic molecule, preferential photodecomposition takes place. This is demonstrated by the change of the isotopic composition (enrichment) of the undissociated SF_6 molecules and of products formed as a result of dissociation.

Much information about isotope separation by dissociation has been obtained using multiple photon absorption of IR radiation for a large number of polyatomic molecules, including BCl_3 (refs 55, 62, 63), SF_6 (refs 56, 64, 65), OsO_4 (ref. 66), CCl_4 (ref. 67), SiF_4 (ref. 63), $\text{C}_2\text{F}_2\text{Cl}_2$ (ref. 63), CH_3NO_2 (ref. 68), MoF_6 (ref. 69), $\text{H}_2\text{Cl}_2\text{C}_2$ (ref. 70), H_2CO (ref. 71), SeF_6 (ref. 72), CF_3I (refs 73, 74). The dissociation of SF_6 molecules, in particular, has been widely investigated. A detailed description of the method of multi-photon IR laser photochemistry is given in refs 4, 5, 75.

The abundance of vibrational-rotational transitions in a polyatomic molecule (which increases sharply after absorption of several IR photons) is the main reason for this ready absorption of many photons up to the dissociation limit. There is no such multiple absorption of IR photons in simple (two- or three-atomic) molecules where the number of possible vibrational-rotational transitions is limited. An excited polyatomic molecule absorbs IR photons by many different vibrations which leads to a strong excitation of a large number of (perhaps all) vibrational degrees of freedom. In this case the high vibrational temperature can be assigned to the excited molecules (thermal model^{75,76}), and its dissociation can be described by the well known theories of monomolecular reactions⁷⁷.

An important modification of this method is the scheme shown in Fig. 3d, when dissociation is carried out by two pulses of IR radiation at different frequencies⁷⁸. In this way it is possible to separate the functions of isotopically selective excitation of a molecule and the dissociation of selectively excited molecules. For selective excitation of a molecule, radiation of a finely tuned frequency ω_1 and intensity is required. Dissociation of selectively excited molecules requires intense radiation of only roughly controllable frequency ω_2 . In this case, isotopic selectivity of dissociation is enhanced as strong non-resonant radiation at frequency ω_2 does not perturb the resonant transition at frequency ω_1 . This method is particularly important for isotope separation of heavy elements. Recent experiments⁷⁹ on OsO_4 molecules have shown that it is possible to obtain a separation selectivity of about 1.6 using two-frequency dissociation, provided all the parameters are chosen correctly; for one-frequency dissociation, however, when a natural mixture of isotopic molecules is irradiated, no isotopic selectivity is obtained.

Recent experiments^{80,81} on multi-photon dissociation of UF_6 molecules by $16\text{ }\mu\text{m}$ radiation pulses of a CF_4 -laser, that is at the frequency of the vibrational band ν_3 of UF_6 , were carried out according to the simplest scheme of dissociation in a single frequency IR field (Fig. 3c). Dissociation characteristics (threshold energy, yield) resemble in many respects the corresponding characteristics for SF_6 . The next obvious step is to obtain the required dissociation selectivity (about 4) using the more delicate schemes of dissociation shown in Fig. 3b,d. A more reliable way of increasing the UF_6 dissociation selectivity is to use dynamically cooled UF_6 to provide distinct resolution of vibrational-rotational absorption lines of $^{235}\text{UF}_6$ and $^{238}\text{UF}_6$ (ref. 83).

Photochemical methods

So far, the possibilities of photochemical isotope separation have been shown by using the excitation of atomic and molecular electronic states and of molecular vibrational levels. However, in spite of the optimism of the early research, the

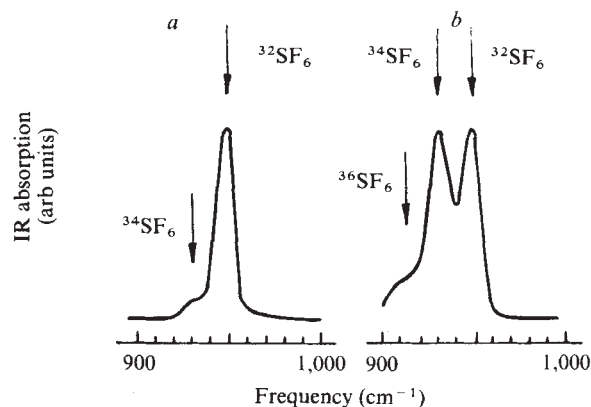


Fig. 4 The infrared spectra of SF_6 before (a) and after (b) irradiation by focused pulses of TEA CO_2 laser. The number of laser shots was chosen so as to equalise the concentrations of $^{34}\text{SF}_6$ and $^{36}\text{SF}_6$ (ref. 56).

actual advances made seem much less impressive than for the photophysical (photoionisation and photodissociation) methods discussed above.

The chemical reactions of electronically excited atoms and molecules have been studied for many years—especially before the application of lasers. The photochemical isotope separation of Hg excited by the 253.7-nm resonance line has been successfully demonstrated with a variety of reagents^{18–24}. For example, Pertel and Gunning²³ were able to enrich ^{202}Hg from 30% of its natural abundance to 85%. The kinetics of the photochemical reactions of Hg are so complex that even the most extensive work does not yield a complete mechanism. Successful experiments on isotopic separation of nitrogen⁸⁴ (or resonance lamp for excitation of NO) and carbon⁸⁵ (iodine resonance lamp for excitation of CO) have subsequently been carried out. An enrichment factor of ~ 4 – 6 has been achieved.

Laser sources for photochemical isotope separation have many obvious advantages over incoherent sources. The broad tunability and high ultimate resolution of lasers give a comparatively free choice of absorption lines, while permitting the highest possible selectivity. This makes the process of photochemical separation of isotopes much easier. It is basically impossible to carry out photophysical experiments of LIS with the usual noncoherent sources of light, because their low radiation temperature means they cannot sufficiently populate the intermediate quantum states and hence cannot provide the effective multi-step process of excitation.

Several successful experiments on laser photochemical enrichment have been reported. The most interesting results have been obtained in experiments on photochemical separation of ^{35}Cl and ^{37}Cl through isotopically selective electron excitation of ICl molecules by cw dye laser radiation^{86–88}. For example, in the case of selective photo-addition of ICl to acetylene, an enrichment factor of ^{37}Cl of 100 was obtained⁸⁸, in the photoproduct $\text{C}_2\text{H}_2\text{ICl}$. Both stable chlorine isotopes can be enriched to more than 97% purity in a single step, starting from natural samples. This is a most promising result in isotopically selective electronic photochemistry.

The rate of a chemical reaction may be substantially enhanced by vibrational excitation of the reactant molecules, and it was suggested as a method for LIS in 1963 (ref. 89). However, the first successful experiment was not until 10 years later⁹⁰ when ^{35}Cl was enriched by enhancing the reaction of Br atoms with vibrationally excited H^{35}Cl . An HCl-pulsed chemical laser was used to excite HCl from $\nu = 0$ to $\nu = 1$ and to $\nu = 2$. For equal pressures of Br and HCl a separation factor of 2 was observed. Recently, carbon isotopic enrichment was also observed in the reaction of vibrationally excited CH_3F molecules with bromine atoms⁹¹. Although the observed enrichment factor was small, this experiment is interesting from the point of view of using a cw IR laser for LIS.

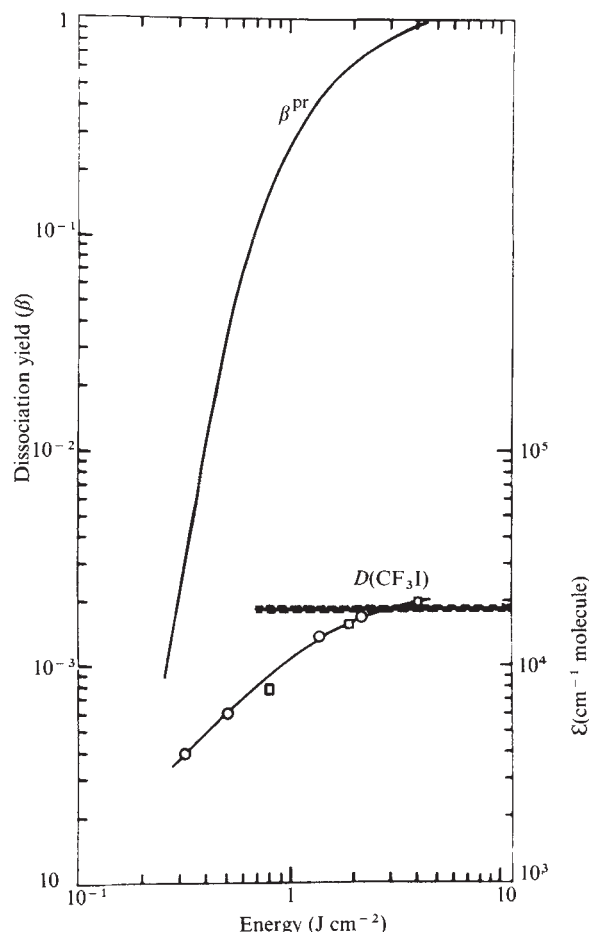


Fig. 5 The dependence of the average energy $\bar{\epsilon}$ absorbed by CF_3I molecule (curve 1, right scale) and of the primary dissociation yield at 0.2 torr CF_3I (curve 2, left scale) on the energy of the CO_2 laser pulse⁷⁴.

Pumping of BCl_3 molecules in a mixture of $\text{BCl}_3 + \text{H}_2\text{S}$ (or D_2S) by a focused, pulsed CO_2 laser, which probably excites high vibrational levels, caused separation of boron isotopes⁹². Clearly this experiment is intermediate between single-photon IR photochemistry in a weak field⁸⁹⁻⁹¹ and multiple photon IR photochemistry in an intense field^{55,56}.

Potential advantages of LIS

The laser method of isotope separation has several potential advantages over the more traditional methods (see ref. 93).

(1) High selectivity of elementary processes of separation. The separation selectivity on one stage α in most traditional methods is very small: $\alpha - 1 \ll 1$. Therefore, many stages of separation N are needed to obtain a high enrichment factor $K = \alpha^N \gg 1$. Laser methods provide $\alpha \gg 1$, which reduces the number of separation stages in most cases to only one or two.

(2) Possibility of extracting the desired isotope leaving other isotopes untouched. When the element has a few isotopes with intermediate masses, we can extract only the desired isotope using a tunable laser leaving other isotopes untouched. This is of particular importance when enriching rare and/or intermediate isotopes.

(3) Low energy consumption. LIS methods consume $\sim 10^2 - 10^3$ eV per separated atom (at 1–10% laser efficiency). Energy consumption per separated atom in most traditional methods is $10 - 10^4$ times higher.

(4) Short starting time. Steady-state operating conditions for most laser methods are achieved immediately. The starting period for traditional methods can be at least several months.

(5) Possibility of running remote separation process using only laser radiation. The absence of traditional separating elements in laser methods leads to minimal contact of the enrichment material with the equipment surfaces, and hence to minimal contamination. This is particularly important for the separation of radioactive isotopes and for the management of radioactive waste of nuclear reactors.

(6) Universality. The efficiency of most traditional methods depends on the mass of the product enriched. Most laser methods can be used with almost equal efficiency for isotope separation of any element, whether light or heavy.

Experiments on a practical scale

However, not all laboratory-scale LIS methods are promising for industrial isotope separation. For industrial scaling the method should have at least two features: first, generation of laser radiation at a power level ranging from 1 kW to 1 MW (depending on the required productivity of the separation set-up). Second, laser equipment which is economical and sufficiently reliable during use.

These two requirements limit the number of LIS experiments that can be carried out on an industrial scale using existing lasers. From this point of view the most promising method is based on isotopically selective dissociation of polyatomic molecules in an intense IR field. The simplest and inexpensive gas flow TEA CO_2 lasers, with total efficiency of 5–10% and average power of up to 1 kW may be used. Initial experiments⁹⁴ on isotope separation with a pulsed CO_2 laser of average power (up to 1 kW) and high repetition rate (up to 180 Hz) have shown that comparatively high isotopic selectivity of dissociation can be obtained in these conditions.

Furthermore recent experiments^{74,95} with CF_3I show that the following theoretical parameters of multi-photon isotopically selective dissociation can be realised: (1) minimum energy consumption equal to the dissociation energy of the weakest molecular bond (2 eV); (2) ultimate (almost 100%) dissociation yield of molecules in the irradiated volume at moderate energy flux of IR radiation; (3) high selectivity of dissociation (>30) at comparatively high (5–10 torr) total pressure of gas; (4) significant extraction (about 50%) of the chosen rare isotope (^{13}C) without substantial decomposition ($<2\%$) of the remaining molecules ($^{12}\text{CF}_3\text{I}$); (5) elimination of secondary photochemical processes which could destroy the high selectivity of primary dissociation. The key characteristics of multi-photon dissociation of CF_3I molecules, which are most important for carbon isotopic separation on a practical scale, are shown in Fig. 5, which shows the dependence of the primary dissociation yield of CF_3I molecules on the energy of the CO_2 -laser pulse, as well as dependence of the average energy $\bar{\epsilon}$ absorbed by the CF_3I molecule on the radiation energy. Clearly when the absorbed energy $\bar{\epsilon}$ is greater than the dissociation energy D , almost all the CF_3I molecules are dissociated.

These results show the potential of the development of an installation for isotope separation based on a CO_2 laser. The simplicity and technological advantages of the LIS method will ensure its early industrial application.

Isotopes for nuclear energy

The isotopic enrichment of ^{235}U for power generation in nuclear reactors is the most economically important objective of LIS. World fuel requirements for nuclear reactors between now and the year 2000 are estimated to be ~ 6 M tons of U_3O_8 . Enrichment cost estimates for processing this ore amount to ~ 150 billion US \$ (refs 3, 96). An efficient laser enrichment scheme could cut these costs dramatically. Several laboratories are therefore experimenting with various schemes for LIS of uranium. As mentioned above, both the atomic process of uranium LIS³⁸⁻⁴² based on photoionisation and the molecular process based on photodissociation of UF_6 molecules⁸⁰⁻⁸³ are being investigated very extensively.

Large quantities of D_2O are needed for heavy water nuclear reactors, which can use natural uranium fuel. The current world

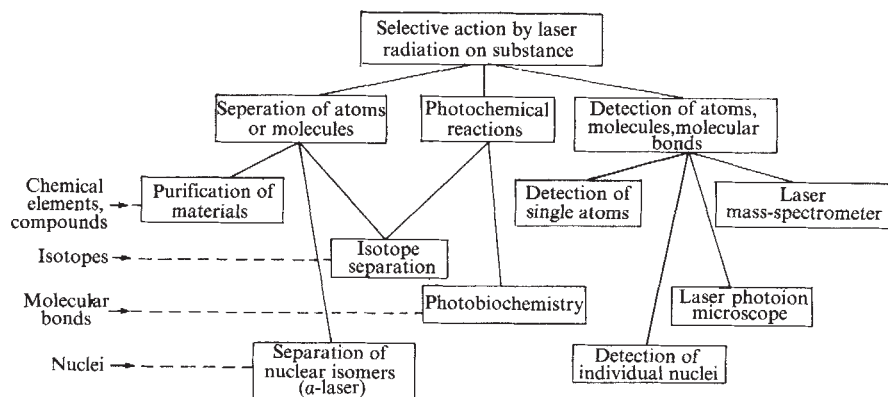


Fig. 6 Types of selective photophysical and photochemical laser processes and their applications.

production capacity is $>10^3$ ton yr^{-1} . The 800 tons of D_2O required for a 1 GW power reactor cost $\sim \$100$ m and account for 15–20% of the total capital cost. Canadian engineers estimate that they will need more than 4×10^3 ton yr^{-1} for their reactors⁹⁷. This means that new effective methods of hydrogen isotope separation must be found. However, it should be kept in mind that as the present production costs per atom of D are 1,000 times less than those for ^{235}U , it will be much more difficult to develop an economically viable process for D. The very low content of deuterium (1/5,000) in natural water demands rather high separation selectivity ($\sim 1,000$) for economical LIS, that is several hundred times more than for uranium. Nevertheless, the possibility of hydrogen LIS by photopredissociation of HDCl using tunable UV laser radiation^{97–99}, and by multi-photon dissociation of polyatomic molecules by CO_2 IR laser radiation¹⁰⁰ is under investigation.

In the future we must also consider applying LIS methods to produce monoisotopic materials of isotopes with low neutron capture cross-section for instance titanium isotope separation¹⁰¹.

Conclusion

It would be difficult to compare in detail the different methods of laser isotope separation without considering specific isotopes or classes of isotopes. Comparison of the methods under development with those already existing is especially complicated. Laser methods are clearly better for separating isotopes that cannot be satisfactorily separated by existing methods, that is less abundant isotopes and the transuranium elements. Major technological progress in the separation of many stable isotopes in comparatively moderate quantities seems to be imminent. In the case of isotopes needed in large quantities for nuclear energy (uranium, deuterium), the situation is still somewhat uncertain. When comparing separation methods for specific isotopes we must keep in mind the level of existing laser technology. In all cases the development of large scale LIS is limited by the energy of present lasers. We need average laser powers within 10^3 – 10^4 W to produce only 1 ton yr^{-1} . In fact, progress in laser technology may change the situation rather drastically. An accurate prediction of the most feasible method of future large-scale laser separation of isotopes cannot be made with certainty given the present state of laser technology.

It is appropriate to emphasise that the selective photophysical and photochemical methods for laser isotope separation will find *fundamental and practical* application well outside the isotope separation problem. With laser radiation it is possible to use various photoprocesses (separation, chemical reactions, detection of atoms, molecules and molecular bonds) selectively in substances at atomic-molecular level with respect to various chemical elements, isotopes, nuclear isomers, molecular stereoisomers, *ortho*- and *para*-molecules, and so on^{102–104}. We are essentially dealing with a new approach to the technology of materials at the atomic and molecular level when one can use

laser radiation to manipulate atoms and molecules of a particular type directly—one can collect macroscopic amounts of matter 'atom by atom' and 'molecule by molecule'.

Besides selective chemical reactions and selective separation of substances at the atomic-molecular level, the process of selective detection of atoms, molecules and molecular bonds is very important. The various processes of selective laser photophysics and photochemistry are summarised in Fig. 6. Isotopically selective photophysical and photochemical processes are currently progressing very rapidly; these and their less evident applications are reviewed in ref. 104. Selective laser photoprocesses open new possibilities to related subjects, such as nuclear physics, spectroscopy, molecular biology, chemical technology and biochemistry. Some of these processes, such as laser identification of nucleotide sequences in DNA, and laser selective biochemical processes are becoming as important as isotope separation by lasers.

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articles

VLBI observations of hot spots in the lobes of distant radio sources

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In a VLBI experiment, using the Westerbork synthesis radio telescope as a total power array and the Effelsberg 100-m dish, 'hot spots' with sizes $\leq 0.15''$ have been detected in the lobes of several 3CR radio sources of high redshift. The individual lobes of double sources and the hot spots within them may have been smaller at earlier epochs.

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MOST powerful extragalactic radio sources with normal radio spectra consist of two extended lobes of emission more or less symmetrically disposed with respect to the parent galaxy or quasar. Often there is also a weak compact component coincident with the optical object. In several cases high resolution observations have indicated the presence of one or more regions of enhanced surface brightness, usually near the outer edges of the radio lobes. It is generally agreed that such 'hot spots' represent regions where radio-emitting electrons are accelerated and supplied to the extended regions of the radio lobes, and several different theories have been put forward to

explain their origin (for example, regions where beams of relativistic particles or low-frequency electromagnetic waves impinge on the intergalactic medium¹; regions of plasma turbulence²; or supermassive objects ejected from the nucleus^{3,4}). Detailed studies of hot spots are clearly important for an understanding of the evolution of radio sources.

Previous investigations of the statistical properties of hot spots in 3CR sources have been carried out using the techniques of aperture synthesis and of interplanetary scintillations (IPS). From synthesis observations at 5 GHz it has been reported⁵ that the fractional source intensity contributed by hot spots correlates well with total source luminosity at 178 MHz, such that hot spots account for nearly all the emission in the most luminous sources. Observations of IPS at 81.5 MHz indicate⁶ that the physical sizes of hot spots may increase with increasing source luminosity. But because of the strong correlation between luminosity and redshift in the 3CR sample, the properties of hot spots noted above correlate as well with redshift as with luminosity, and if the physical sizes of hot spots do not change with redshift the IPS results can also be interpreted to imply⁷ a value of q_0 , the deceleration parameter, in the range $0.5 < q_0 < 2$. However, the properties ascribed to hot spots in sources at low and high redshifts may not always refer to similar physical features in their brightness distributions⁸. This is particularly true if the individual lobes of radio sources were physically smaller at earlier epochs. As the lobes of many radio sources at high redshifts ($z \geq 0.5$) are often only 1 or 2 arcs in angular extent the existing aperture synthesis and IPS observations cannot indicate whether the observed components are true hot spots without any extended lobes around them or whether they have finer structure in them attributable to hot spots. Angular resolution considerably better than 1 arc s is therefore necessary to answer this important question.

We report here observations of the lobes of high luminosity 3CR sources carried out with a very-long-baseline interferometer (VLBI) system using the Westerbork synthesis array and the Effelsberg 100-m antenna. Compact components containing significant fractions of the total flux densities in sizes ≤ 0.15 arc s have been detected in the lobes of several radio sources of large redshift. These compact components have luminosities comparable to those of hot spots in nearby sources of similar total luminosity. There is, however, some indication that the physical sizes of these hot spots may have been smaller at earlier epochs, contrary to the inference drawn from the IPS results.

The source sample

A total of 35 radio sources was observed. This included 27 quasars: all those (except 3C191 and 3C207) in the 3CR sample of 200⁹ that have (1) redshift $z > 0.5$; (2) radio spectral index $\alpha < -0.5$ (defined as $S_{\nu} \propto \nu^{-\alpha}$) throughout the frequency range of 80–5,000 MHz; and (3) no known low-frequency cut-off down to 38 MHz. The other eight sources observed were galaxies satisfying the above criteria, with the redshift estimated in most cases from the optical magnitudes. The sources have a luminosity $\geq 9 \times 10^{26} \text{ W Hz}^{-1} \text{ sr}^{-1}$ at 178 MHz and nearly all of them are known to be standard double sources with component separations > 1 arc s.

The interferometer system

The Effelsberg 100-m telescope and the 1.6-km Westerbork synthesis radio telescope used in total power mode formed the elements of the VLB interferometer for these observations at 1,417 MHz (see Table 1). The observations took place between 15.00 UT on 31 March and 15.00 UT on 2 April in 1978. The polarisation was linear in PA 0° on the sky.

Total power operation at Westerbork was achieved by coherently adding the signals from the Y dipoles of the 14 telescopes in the array after IF conversion to baseband. The added baseband signal was fed directly to the format unit of the VLBI recording terminal. A full description of the adding

Table 1 Elements of the interferometer

	Antenna diameter (m)	System temperature at zenith (K)	Sensitivity (K Jy^{-1})
Effelsberg	100	75	1.5
Westerbork	93*	85	1.4

*Equivalent diameter; the Westerbork array has 14 dishes each of diameter 25 m.

system is given elsewhere¹⁰. Steering of the telescope in delay and fringe rate (after phase offsets for the individual telescopes had been determined) was carried out in the last IF stage—the normal mode of operation for the Westerbork telescope. The fringe stopping centre, the delay centre, and the centre of the fan beam were identical on the sky. The orientation and half-power width of the fan beam are functions of the hour angle and declination of the source; at zenith on the meridian the beam width is 22 arc s east–west and 22 arc min north–south.

Up to four short observations, each of ~ 10 m duration were made for each source at different hour angles within the ± 6 h range at Westerbork. Observations of calibrators were interspersed at regular intervals throughout the period. In cases where the width and orientation of the Westerbork fan beam encompassed both lobes of the source, a single observation of the whole source was made. In other cases observations of each lobe were made independently.

The Effelsberg–Westerbork interferometer is 1.26 M λ in length at the observing frequency, giving a fringe spacing of ~ 0.16 arc s. The length of the baseline changes very little with hour angle, but the orientation changes considerably, allowing one to say whether any particular source is non-circular in structure.

The recording of the signals was carried out with the standard Mk IIC VLBI system with a bandwidth of 2 MHz and the subsequent cross correlations and data reduction were done at the Max Planck Institute for Radio Astronomy. A hydrogen maser oscillator at Effelsberg and a rubidium oscillator at Westerbork provided the time and frequency standards.

Calibration of the data

The correlated flux density scale was calibrated using the sources NRAO140, 4C39.25, OQ208, 3C345 and 3C454.3. Table 3 lists the calibrator sources, the number of times they were observed, the total flux density as measured at Effelsberg during the observations and the flux density in compact components. In NRAO140 and OQ208 the compact components supply the total flux density. Measurements of the larger scale structure in 3C345 and 3C454.3 by Davies *et al.*¹¹ were used to remove the contribution of these steep-spectrum resolved components to the total flux density. Available spectral information on 4C39.25 was used to remove the contribution of a steep-spectrum component. The calibration factor for all five sources has an r.m.s. scatter of $\sim 6\%$. Removing OQ208 reduces the r.m.s. scatter to $\sim 2\%$; this implies that the measured flux for OQ208 may be too low. The measured r.m.s. scatter in amplitude from one scan to another for the individual calibrators is $\sim 4\%$.

Checks on the calibrator sources indicated that there was no loss in fringe amplitude due to phase drifts in the oscillators (primarily the rubidium) on time scales of 10 m. Consequently,

Table 2 Geocentric coordinates of the observatories

Observatory	X (km)	Y (km)	Z (km)
Effelsberg	4,033.778	−486.995	4,900.529
Westerbork	3,828.674	−443.226	5,064.916

The X-axis points to latitude 0°, longitude 0°; the Y axis to latitude 0°, longitude 90°; and the Z axis to latitude 90°.

each observation on the extended sources was integrated coherently for the duration of the observation, typically 10 m. The r.m.s. noise in correlated flux density for a 10 m coherent integration was observed to be 3 to 4 mJy, allowing reliable detections of compact components as weak as 15 to 20 mJy.

The X and Y components of the baseline were determined by least-squares fitting to the fringe rates for the calibrators, and a crude estimate of the Z component was made from the delay function corresponding to the 2 MHz recorded bandwidth. The X , Y , Z parameters of the individual telescopes are given in Table 2. The r.m.s. scatter in the fringe rates of the calibrators about zero residual fringe rate is ± 1 mHz assuming the best fit baseline. This means that the pointing of the beam centre is known to that accuracy in the fringe rate variable. This is sufficient to distinguish, in many of the sources observed, between a compact component at the centre of the beam and one in an outer lobe.

The width and orientation of the Westerbork beam were calculated for each observation. In cases where components were detected away from the beam centre their flux densities were scaled according to their positions in the beam.

Correlated flux density from hot spots and nuclear components

The correlated flux density for each observation was determined from the fringe frequency spectrum obtained by transforming all the data for that observation. A comparison of the residual fringe frequency for each significant peak in the spectrum with the expected values for components in the individual lobes and at the location of the optical object enabled unambiguous identification of the observed VLBI components in most sources in which the lobes are separated by ≥ 10 arc s.

Correlated flux density exceeding a signal-to-noise ratio of 5 was detected in all the sources observed except 3C41 for which only a single 10 m observation was available. The results for sources in which at least one hot spot is likely to have been detected in the outer lobes are presented in Table 4a. The observed components have been identified in Table 4 by the letters A, B or N which refer respectively to the western lobe, the eastern lobe and the nuclear component associated with the optical object. Other relevant information listed in Table 4 is the optical identification, the redshift, the angular separation between the outer lobes, θ_{AB} (taken from ref. 12 and references therein), the measured total flux density at 21 cm and the flux density in the nuclear component at 6 cm as measured by the Cambridge 5 km telescope. A blank in column 6 implies that a nuclear component was not detected at 6 cm and a question mark implies that flux density in the nuclear component cannot be determined unambiguously. The number of different hour angles at which the VLBI observations were made is given in column 7 and the maximum correlated flux density for each component is listed in column 8. A more detailed table giving the measurements at each observed U, V point will be published elsewhere. The errors quoted in column 8 are a quadratic

combination of 4% of the total flux density and the r.m.s. noise for that observation.

Sources for which the observed VLBI components are likely to be associated with nuclear components are listed in Table 4b. As can be seen from Table 4a and b the correlated flux density measured by us in the nuclear components at 21 cm is in general less than or only slightly more than that measured at 5 GHz with the Cambridge 5-km telescope. This agrees with the generally accepted result that most nuclear components have flat or inverted radio spectra at these frequencies. We also find that the variation in correlated flux density with hour angle for the nuclear components is in general small, implying that these components are unresolved with the present baseline.

In a few sources, mainly those for which the overall radio structure is not well determined, it is not possible to make unambiguous identifications of the observed VLBI components with known features in their brightness distributions. In the case of 3C43 and 3C454 the correlated flux density shows little variation with hour angle and corresponds to $\sim 26\%$ and $\sim 16\%$ of the total flux at 21 cm, respectively. The overall sizes of the sources are < 1 arc s and the total spectra are straight between 38 MHz and 5 GHz with no sign of a flattening at high frequencies¹³. The observed VLBI components in these sources are therefore unlikely to be associated with flat spectrum nuclear components.

In the case of 3C186 only the northern component (A) of the 100 arc s double¹⁴ was observed. This component lies close to the QSO and could itself be double with a component separation of 1.8 arc s (ref. 15). It is uncertain whether the weaker southern lobe is physically related to the QSO. The maximum observed correlated flux density is $\sim 22\%$ of the total at 21 cm and is unlikely to arise in a flat spectrum nuclear component as there is no observed flattening of the total spectrum at high frequencies¹³.

The overall size of 3C216 is < 1 arc s and the total spectrum which seems to flatten out at frequencies above 1.4 GHz (ref. 13) is consistent with all the correlated flux arising in a flat spectrum nuclear component.

3C245 ($\theta_{AB} = 5''$) has a D2 type structure¹⁶; the component B being coincident with the QSO¹². The correlated flux density corresponds to $\sim 47\%$ of the total and could be associated with component B.

A map of 3C380 at 15 GHz (ref. 17) shows this source to be a double with a component separation of ~ 1.2 arc s, the stronger component possibly coinciding with the QSO. The VLBI component seems to be partly resolved and could be associated with the nuclear component.

Comparison with Cygnus A

It is interesting to compare the properties of hot spots in sources of large redshift with those in the well observed source Cygnus A, which is a relatively close radio galaxy ($z = 0.056$) with an intrinsic luminosity similar to that of the QSOs observed here. Aperture synthesis observations at 5 GHz indicate¹⁸ the presence of four hot spots in Cygnus A with angular sizes between ~ 1 and 2 arc s. From the available spectral information it is estimated that at 1.4 GHz the hot spots together should account for about 19% of the source flux density, most of which is contributed by two most prominent hot spots numbered A ($\sim 7\%$) in the Np lobe and D ($\sim 10\%$) in the Sf lobe¹⁸. These spots have physical sizes between about 2 and 3 kpc. Higher resolution observations at 15 GHz (ref. 19) indicate little significant structure on a more compact scale.

From the present VLBI observations it is not possible to estimate the exact shapes of the hot spots or the fractional source flux density which they contain. As the length of the projected baseline does not change significantly over the hour angle range of these observations, the interferometer is sensitive to similar angular scales in most sources. At the mean projected baseline of $1.2 \times 10^6 \lambda$ the normalised fringe visibilities of gaussian structures with half-intensity diameters of 0.05, 0.1 and 0.15 arc s would be about 0.74, 0.30 and 0.07, respectively. The

Table 3 The calibrators

Source	No. of scans	Measured total flux density (21 cm)* (Jy)	Unresolved flux density (Jy)
NRAO 140	2	3.66	3.66
4C39.25	2	2.53	2.05
OQ208	5	0.81	0.81
3C345	2	7.39	6.77
3C454.3	1	10.86	10.23

*For an assumed sensitivity of $1.51 \pm 0.06 \text{ K Jy}^{-1}$. This value was derived from the measured antenna temperatures at Effelsberg for all the 3CR sources in the sample and using the total flux densities in Bridle *et al.*²⁶.

Table 4 *a*, Sources with hot spots; *b*, sources in which only nuclear components were detected

Source	Optical identification	Redshift	θ_{AB} (arc s)	S_{tot} (21 cm) (Jy)	S_{nuclear} (6 cm) (mJy)	No. of scans	Maximum S_{corr} (21 cm) (mJy)
<i>a</i>							
3C6.1	G	(0.75)	26	3.32	20 ± 10	1	72 ± 5 (component B)
9	Q	2.012	10	1.93	—	3	28 ± 4 (B or N)
13	G	(0.5)	28	1.80	—	3	140 ± 7 (A) 145 ± 7 (B)
43	Q	1.459	<1.5	2.79	?	3	773 ± 31*
55	G	(0.75)	72	2.38	—	3	62 ± 5 (A)
68.1	Q	1.238	46	2.45	—	4	34 ± 5 (A)
175	Q	0.768	48	2.45	40 ± 8	2	20 ± 4 (B)
181	Q	1.382	6	2.29	—	3	179 ± 8 (A) 332 ± 14 (B)
186	Q	1.063	100	1.35	?	4	291 ± 12 (A)*
196	Q	0.871	5	13.84	—	4	532 ± 22 (A more likely than B)
205	Q	1.534	16	2.29	30 ± 10	3	317 ± 13 (A)
254	Q	0.734	13	2.83	—	3	112 ± 6 (A) 64 ± 5 (B)
263	Q	0.652	44	3.07	130 ± 20	2	264 ± 11 (B) 173 ± 8 (N)
268.4	Q	1.40	10	2.10	50 ± 20	4	503 ± 21 (A)
270.1	Q	1.519	12	2.59	?	4	290 ± 12 (B + N)
275.1	Q	0.557	14	2.89	—	3	70 ± 5 (A) 116 ± 6 (B)†
280	G	(0.5)	13	5.00	—	4	356 ± 15 (A)
280.1	Q	1.659	22	1.38	55 ± 10	3	23 ± 4 (A) 45 ± 5 (N)
288.1	Q	0.961	7	1.51	—	3	39 ± 4 (A) 44 ± 4 (B)
330	G	0.549	56	6.62	—	1	46 ± 4 (A more likely than B or N)
352	G	(0.5)	10	1.84	—	3	49 ± 5 (A, B or N)
432	Q	1.805	13	1.56	—	3	45 ± 4 (B more likely than A)
454	Q	1.757	<1.3	1.96	?	3	333 ± 14*
455	Q	0.543	3.2	2.76	?	3	75 ± 5 (A or B)
<i>b</i>							
3C200	G	(0.5)	17	2.02	88 ± 15	4	19 ± 4
204	Q	1.112	31	1.30	40 ± 10	4	82 ± 5‡
208	Q	1.109	11	2.42	54 ± 5	1	79 ± 6
212	Q	1.043	9	2.50	310 ± 50	1	85 ± 6
216	Q	0.67	0.8	3.88	?	2	554 ± 23*
220.2	Q	1.157	8	1.75	60 ± 15	3	118 ± 6†
245	Q	1.029	4.6	3.18	1,200 ± 100	2	1,485 ± 60*
334	Q	0.555	48	1.96	170 ± 20	2	180 ± 8
336	Q	0.927	22	2.48	30 ± 10	3	19 ± 5
380	Q	0.691	1.2	13.63	?	3	4,033 ± 160*

*See text. †May include some contribution from a nuclear component. ‡May include some contributions from A and B.

maximum correlated fluxes listed in Table 4 are therefore in most cases likely to be lower limits to the flux densities contained in hot spots while the angular diameters of the hot spots are likely to be ≤ 0.15 arc s, which corresponds to ≤ 1.3 kpc at $z = 1$ (for $H = 50 \text{ km s}^{-1} \text{ Mpc}^{-1}$ and the Einstein–de Sitter world model), typical of the sources observed.

It is clear from a histogram of the normalised maximum visibility (γ_{max}) for all the sources observed here (Fig. 1a) that in over one-third of them the hot spots are responsible for at least 5% of the source flux density, and in this respect are not very different from those in Cygnus A. The physical sizes of these hot spots are, however, likely to be smaller than those of Cygnus A. Hot spots of size > 2 kpc at $z > 0.5$ would, in fact, have been completely resolved out in the present experiment. It is therefore possible that hot spots similar to those in Cygnus A are even more common in high redshift sources than is apparent from the present number of detections.

In view of the limited U, V coverage of the observations it is important to ask if the observed visibilities could arise from highly non-gaussian brightness distributions such as uniform disks or linear structures with sizes > 0.15 arc s. A circular disk in our case would have a secondary maximum in the visibility curve for a diameter of 0.3 arc s with $\gamma \sim 0.13$. As seven sources have $\gamma_{\text{max}} > 0.13$, such distributions are unlikely to be true in general. Higher visibilities at the secondary maximum are possible for linear brightness distributions but the values of γ would be strongly dependent on the hour angle of observation due to projection effects. Only in three of the 13 sources with $\gamma_{\text{max}} > 0.05$ was the ratio of γ_{max} to γ_{min} found to be > 2 and only

in one case > 3 . As the sources were observed typically at three different position angles differing by over 90° on the sky, such highly elongated components seem to be unlikely unless their maximum dimensions are appreciably smaller than the fringe separation of the interferometer.

The minimum equipartition energy requirements in the hot spots of Cygnus A have been estimated¹⁸ to be of the order of 3×10^{57} erg. It was also shown by Hargrave and Ryle¹⁸ that for inferred equipartition magnetic field of $\sim 3 \times 10^{-4}$ G the radiation lifetime of 5 GHz electrons is only $\sim 5 \times 10^4$ yr and that continuing acceleration of electrons must therefore take place in the hot spots and the particles must diffuse out of them rather quickly. These arguments are also valid for the hot spots seen in more distant sources. As the total luminosities of many of the hot spots reported here are comparable with those in Cygnus A, the magnetic field should scale as $V^{-2/7}$, whereas the minimum total energy and the electron lifetimes should scale as $V^{3/7}$, where V is the volume of the hot spots. Because the volumes of the hot spots reported here could be more than 10 times smaller than Cygnus A the electron lifetimes at radio frequencies would be even smaller than in the case of Cygnus A.

Cosmological implications

There is considerable evidence from the observed angular size–redshift relationship for QSOs (see ref. 20) and the angular size–flux density relationship^{21,22} that the lobe separations of double radio sources were smaller at earlier epochs. It is important to know if such evolutionary effects may also be present in the sizes of individual lobes of radio sources and of hot

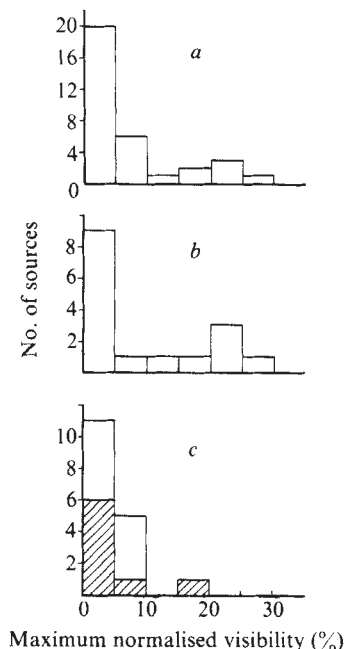


Fig. 1 The distribution of maximum observed correlated flux densities from hot spots, normalised with respect to the total source flux densities, in: *a*, all the sources; *b*, sources with $z > 1$ and *c*, sources with $z < 1$. Sources in which hot spots were not detected have been included in the first bins (0 to 5%). 3C216 and 3C380 have not been included in the histograms and galaxies are shown hatched.

spots within them. A plot of individual lobe sizes with redshift for the 3CR sources shows⁸ that at high redshifts ($z > 0.5$) most sources have lobe sizes of only 1–2 arc s which is considerably smaller than expected in reasonable world models if the lobes had similar physical sizes to those at lower redshifts. Our detection of compact hot spots similar in intensity to those in Cygnus A in many high redshift sources implies that the component sizes measured by the Cambridge 5 km telescope for these sources refer to the lobes and not to the hot spots within them. It is therefore likely that the physical sizes of individual lobes of double radio sources undergo similar evolutionary effects with epoch as the lobe separations.

The detection of hot spots of size ≤ 0.15 arc s in high-luminosity sources also suggests that the strong correlation between the fractional flux in hot spots and total source luminosity suggested by Jenkins and McEllin⁵ for the 3CR sources with Class II²³ radio structure, suffers from insufficient resolution of the synthesis observations. For this correlation Jenkins and McEllin defined hot spots as regions with a size < 15 kpc, which corresponds to an angular size < 2 arc s for redshifts ≥ 0.5 . As most radio sources at $z < 0.3$ have measured lobe sizes > 15 kpc and those at $z > 0.5$ have lobes < 15 kpc (see ref. 8) the 2 arc s resolution of the synthesis observations is insufficient to distinguish the contribution of hot spots from that of the more extended lobes at higher redshifts (or higher luminosities). But due to the criterion used to define hot spots nearly all

the source flux density is considered to arise in hot spots. This could be responsible for much of the observed correlation. Because of the very different angular resolutions it is difficult to compare the fractional flux in hotspots derived from synthesis observations of nearby sources with that detected by VLBI in the present high redshift sample. However, it is interesting that the fractional flux in hotspots at 5 GHz for the 10 sources in the table of Jenkins and McEllin⁵ with $z < 0.1$ varies between 5 and 38%, with an average value of 14%, not significantly different from the VLBI visibilities seen in the high redshift sources.

If the physical sizes of hot spots in Cygnus A are typical of low redshift sources, as is indicated by IPS observations⁶, then the present detection of hot spots with sizes < 1 kpc in several of the high redshift sources suggests that the hot spot sizes may also have been smaller at earlier epochs. Some further support for such a possibility may be seen within the present source sample if the sources are divided into two groups depending on whether these have redshifts greater than or less than 1, as shown in Fig. 1*b, c*. Although the sample is small the values of $\gamma_{\max}$ seem to be higher in the $z > 1$ sample, as might be expected if the hot spots were more compact at higher redshifts. The observations are, however, also consistent with a weak correlation between the fractional flux in hot spots and redshift.

The possible evolutionary effect indicated by the present observations is contrary to the conclusions of Hewish *et al.*⁷ based on IPS observations. Their plot of the inferred angular diameter of hot spots for a sample of 23 strongly scintillating 3CR sources against redshift indicated a strong deficit of sources with diameters < 0.3 arc s at redshifts > 0.7 . This was interpreted by them to imply either that $0.5 < q_0 < 2.0$, or if q_0 is to be smaller, the linear size of hot spots must increase with redshift. But the present VLBI measurements make it doubtful if the angular diameters derived from IPS for high redshift sources refer to true hot spots. This may be seen from a comparison of the VLBI and IPS observations for the eight sources that seem to be common to the two samples at $z > 0.7$ (the IPS data are from ref. 6). Table 6 shows the parameters R and ψ (which are measures of the fractional flux density and the angular diameter of the scintillating component respectively) derived from IPS, together with the values of $\gamma_{\max}$ and $\gamma_{\min}$ in hot spots observed by VLBI.

Based on a new determination of the spectrum of electron density fluctuations in the solar wind, Readhead *et al.*²⁷ have recently pointed out that the published values of R and ψ should be reduced by $\sim 40\%$ and $\sim 25\%$, respectively. The revised values have been used in Table 5. The values of ψ quoted in Table 5 have been further reduced by $\sim 20\%$ to convert the e^{-1} full widths used to define the size in ref. 6 to full widths at halfpower. In six of the eight sources, hot spots with angular diameters ≤ 0.15 arc s contain at least 6–28% of the source flux density. The diameters derived from IPS for these sources, on the other hand, are generally > 0.3 arc s. The angular diameters of the individual lobes as determined directly from synthesis observations with the Cambridge 5-km telescope are in most cases less than or of the order of 2 arc s. As all structures smaller than about 2 arc s contribute to IPS at 81.5 MHz, the diameters inferred from IPS are likely to be suitably weighted means of the hot spot and lobe sizes. The importance of such a blending of source components in interpreting the IPS results was pointed out earlier by Swarup and Bhandari²⁴, but it was noted by Hewish and Readhead²⁵ that the estimated values of R for sources of large z are considerably higher than would be expected if blending were important. The downwards revision by 40% in the values of R , however, makes them consistent with the blending hypothesis.

The present VLBI observations together with the overall source structures determined by aperture synthesis thus suggest that the individual lobes of double sources and perhaps also the hot spots within them were more compact at earlier epochs. Because of the tight correlation between redshift and luminosity in the 3CR sample it is not, however, possible to rule out the dependence of component sizes on absolute luminosity rather

Table 5 Comparison of VLBI and IPS results

Source	VLBI		IPS	
	$\gamma_{\max}$	$\gamma_{\min}$	R	ψ (arc s)
3C43	0.28	0.24	0.51	0.26
3C181	0.22	0.10	0.39	0.47
3C186	0.22	0.09	0.51	0.32
3C196	0.04	0.01	0.33	0.70
3C254	0.06	0.05	0.33	0.64
3C268.4	0.24	0.14	0.51	0.50
3C270.1	0.11	0.05	0.24	0.14
3C280.1	0.02	< 0.02	0.45	0.70

than on cosmological epoch. Observations of much weaker samples to see lower luminosity sources at high redshifts are needed to decide between the two possibilities. High-resolution mapping of compact structures in the radio lobes may also throw light on the physical nature of the evolutionary mechanism.

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Isotopes of tellurium, xenon and krypton in Allende meteorite retain record of nucleosynthesis

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Various mixtures of r-, p- and s-nucleosynthesis products have been observed in the isotopes of tellurium, xenon and krypton extracted from mineral separates of the Allende meteorite. The presence of several isotopically distinct components in these high Z elements and the close association of isotopically normal low Z elements with particular isotopes of the high Z elements suggest that our Solar System may have formed directly from the debris of a single precursor star, approximately concentric with the present Sun.

THE enrichment of heavy xenon isotopes in carbonaceous chondrites is always accompanied by an enrichment of the light, fission-shielded isotopes. It has been suggested that this anomalous xenon might represent a mixture of r- and p-products added to the Solar System from a nearby supernova¹—previously the excess heavy isotopes were widely regarded as fission products, designated CCF as an acronym for carbonaceous chondrite fission². More recent reports of nucleogenetic anomalies in the isotopic compositions of several other elements in meteorites^{3–10} and identification¹¹ of the decay product of extinct ²⁶Al have resulted in three different models of element synthesis shortly before condensation: (1) The anomalous elements and short-lived isotopes might have been generated in remote nucleosynthesis events, such as supernovae^{12,13} or red giant stars^{14,15}, and then trapped in grains which were subsequently incorporated into the early solar nebula. In such a case the short-lived isotopes themselves would not necessarily survive until the grains entered the solar nebula, but the more refractory dust grains might survive conditions in the nebula and be incorporated into meteorites with alien nucleosynthesis products and the decay products of short-lived isotopes. (2) The anomalous elements and short-lived isotopes might have been

generated in a nearby supernova¹⁶. If the shock wave from this supernova caused collapse of the presolar nebula, then the nucleosynthesis event is directly related to the birth of the Solar System and occurred about 4.6×10^9 yr ago. (3) The Solar System might have formed directly from the debris of a single supernova^{17–19}. In this case the anomalous elements and short-lived isotopes were made by the same nucleosynthesis events which produced other elements in the Solar System, and the present Solar System retains the chemical characteristics of the supernova: The Sun accumulated on the supernova core, iron meteorites and cores of the terrestrial planets were formed primarily from elements synthesised in the hot stellar interior, and the outer planets and the carbon phase of chondrites were condensed from elements of the cooler outer layers, the only region which contained low-Z elements.

Two recent reviews^{20,21} suggest that model (2), a nearby supernova, has gained favour as a reasonable working hypothesis. However, the results of analyses on noble gases trapped in the metal phase of iron meteorites²² and in gas-rich xenoliths²³ from the Earth's mantle have been interpreted as supporting model (3). Other tests were suggested for model (3), including its prediction that the acid-resistant carbon which contains anomalous xenon should also contain isotopically anomalous components of other high-Z elements²². Subsequently, Arden⁶ has reported large isotopic anomalies of uranium in acid-resistant minerals of several chondrites, but it was uncertain whether these were generated by stellar nuclear reactions or by chemical fractionation of the precursor elements of ²³⁵U and ²³⁸U. The present study was therefore undertaken to test the prediction of model (3) at tellurium ($Z = 52$), an element with an atomic number near that of xenon ($Z = 54$) and with eight natural isotopes that are only inefficiently produced by fission.

Srinivasan followed procedures developed elsewhere^{24–27} to isolate the following acid-resistant fractions from a crushed sample of bulk Allende weighing 11.66 g. (a) Residue A: Etch-

Table 1 Ratios of stable xenon isotopes

Isotope	Carbon (C-2)				Spinels and carbon (B-1)			Spinels*	AVCC	Air
	800°	1,200°	1,600°	Total	800°	1,600°	Total	Total		
¹²⁴ Xe	0.497 ±0.005	0.564 ±0.008	0.572 ±0.007	0.548 ±0.004	0.476 ±0.006	0.485 ±0.008	0.478 ±0.006	0.355 ±0.018	0.459	0.357
¹²⁶ Xe	0.447 ±0.008	0.458 ±0.007	0.456 ±0.006	0.454 ±0.005	0.417 ±0.005	0.409 ±0.018	0.415 ±0.008	0.348 ±0.024	0.410	0.335
¹²⁸ Xe	8.29 ±0.08	8.42 ±0.08	8.44 ±0.11	8.39 ±0.06	8.29 ±0.06	8.02 ±0.17	8.24 ±0.08	7.97 ±0.24	8.20	7.14
¹²⁹ Xe	140.3 ±0.8	106.1 ±0.6	105.2 ±0.6	115.2 ±0.4	116.6 ±0.5	102.3 ±0.6	113.8 ±0.5	111.4 ±1.6	≈100	98.3
¹³⁰ Xe	16.13 ±0.11	15.90 ±0.09	15.98 ±0.09	15.99 ±0.07	16.28 ±0.09	15.84 ±0.10	16.19 ±0.09	16.55 ±0.28	16.08	15.17
¹³¹ Xe	81.55 ±0.26	82.24 ±0.29	82.21 ±0.37	82.04 ±0.23	81.95 ±0.32	80.86 ±0.62	81.74 ±0.38	81.21 ±1.12	81.7	78.77
¹³² Xe	≈100	≈100	≈100	≈100	≈100	≈100	≈100	≈100	≈100	≈100
¹³⁴ Xe	38.44 ±0.15	45.21 ±0.24	43.92 ±0.17	42.98 ±0.12	38.15 ±0.18	40.87 ±0.18	38.68 ±0.18	31.16 ±0.54	38.2	38.82
¹³⁶ Xe	32.41 ±0.23	42.74 ±0.30	40.97 ±0.20	39.39 ±0.12	32.07 ±0.22	36.13 ±0.29	32.86 ±0.23	21.45 ±0.67	32.1	32.99
¹³² Xe content (cm ³ STP g ⁻¹) × 10 ⁻⁸	1.22	1.94	1.29	4.45	2.60	0.62	3.22	2.17	—	—

* (Amount of isotope in spinels) = (Amount of isotope in B-1) - (Fractional wt of C in B) $\frac{\text{wt of B-1}}{\text{wt of C-2}}$ (Amount of isotope in C-2).

ing bulk Allende in HCl and HF acids for a 6-week period produced residues weighing 0.53% of the bulk sample. Previous studies²⁴⁻²⁷ on Allende residues suggest that carbon, chromite, spinel and an ill-defined mineral Q are the main constituents of residue A. (b) Residue B: Etching residue A in concentrated HNO₃ dissolved Q (refs 26, 27), leaving a residue weighing 0.42% of the bulk Allende sample. (c) Residue C: Refluxing residue B in a mixture of concentrated acids, H₃PO₄ and H₂SO₄, for 2 h dissolved²⁸ the chromite and spinel (and perhaps part of the carbon) to produce residue C, weighing 0.19% of the bulk sample. (d) Dissolution of C: Refluxing residue C for 1 h in a mixture of concentrated acids, H₃PO₄ and H₂SO₄, plus CrO₃, dissolved residue C.

Nucleosynthetic products in xenon and krypton

A high sensitivity noble gas mass spectrometer was used to determine the isotopic compositions of xenon and krypton released by stepwise heating of a 3.1-mg aliquot of residue B, labelled B-1, and a 1.7-mg aliquot of residue C, labelled C-2. Procedures for extraction and analysis of noble gases were as described previously²². The isotopic compositions of xenon and krypton in these residues are presented in Tables 1 and 2, respectively. The xenon in both residues displays the now familiar pattern of enrichment in the light and heavy isotopes.

The part (chromite, spinel and perhaps some carbon) of residue B which dissolved (54% by weight) in producing residue

C is referred to collectively as spinels in Tables 1 and 2. The concentrations and isotopic structures of gases in these spinels were calculated from differences in the isotopic compositions and total gas contents of residues B and C. Superficially the isotopic composition of xenon and krypton in Allende spinels is similar to that reported recently in the high temperature fractions of Murchison residues which were etched in alkaline oxidants to remove organic polymers¹⁵. It was reported that X-ray diffraction of the severely etched Murchison residues showed mainly chromite and minor spinels—minerals which probably constitute the bulk of our Allende spinels. As was observed in the high temperature fractions of these Murchison residues¹⁵, the xenon in Allende spinels is enriched in ¹³⁰Xe and depleted in ¹³⁶Xe, and the krypton in these spinels seems to be enriched in ⁸²Kr and depleted in ⁸³Kr. However, the isotopic composition of Kr in our spinels cannot be distinguished from bulk krypton in carbonaceous chondrites or in air, within the limits of experimental error. A detailed examination of the xenon spectrum in Allende's spinels is instructive in evaluating the model proposed to explain the high temperature xenon in Murchison's spinel—s products trapped in presolar dust grains that were ejected from red giant stars^{14,15}.

The isotopic spectra of xenon in Allende's carbon and spinels are shown in Fig. 1, normalised to xenon in average carbonaceous chondrites (AVCC) in the following manner.

$$g_{132}^i = ({}^i\text{Xe}/{}^{132}\text{Xe})_{\text{sample}} / ({}^i\text{Xe}/{}^{132}\text{Xe})_{\text{AVCC}} \quad (1)$$

Since the *in situ* decay product of ¹²⁹I may comprise part of the

Table 2 Ratios of stable krypton isotopes

Isotope	Carbon (C-2)				Spinels and carbon (B-1)			Spinels*	AVCC	Air
	800°	1,200°	1,600°	Total	800°	1,600°	Total	Total		
⁸⁰ Kr	4.22 ±0.06	3.95 ±0.04	3.92 ±0.07	4.05 ±0.04	4.09 ±0.07	3.87 ±0.04	4.02 ±0.06	3.98 ±0.20	3.92	3.96
⁸² Kr	20.45 ±0.17	19.72 ±0.05	19.32 ±0.03	19.88 ±0.09	20.25 ±0.28	19.75 ±0.17	20.09 ±0.25	20.51 ±0.75	20.14	20.21
⁸³ Kr	20.42 ±0.15	20.11 ±0.11	20.00 ±0.10	20.20 ±0.12	20.17 ±0.10	19.92 ±0.08	20.09 ±0.09	19.88 ±0.36	20.17	20.16
⁸⁴ Kr	≈100	≈100	≈100	≈100	≈100	≈100	≈100	≈100	≈100	≈100
⁸⁶ Kr	30.33 ±0.27	31.44 ±0.15	31.48 ±0.19	30.91 ±0.21	30.55 ±0.14	31.24 ±0.15	30.77 ±0.14	30.49 ±0.59	30.98	30.55
⁸⁴ Kr content (cm ³ STP g ⁻¹) × 10 ⁻⁸	4.13	2.89	3.43	10.45	4.98	2.28	7.26	4.53	—	—

* See Table 1 footnote for method used to calculate amount of each isotope in spinels.

^{129}Xe abundance, this isotope is not used to define the general anomaly pattern of isotopes trapped in Allende's spinels and carbon. Abundances of the other xenon isotopes in carbon and spinels display opposite anomaly patterns. We suggest that these mineral fractions trapped different components of AVCC xenon, that is, carbon is enriched in r and p products and spinels are enriched in s products of the nucleosynthesis reactions which collectively produced the isotopes of bulk xenon in carbonaceous chondrites. It seems highly unlikely that the carbon and spinel fractions are both interstellar grains, or that either the carbon or the spinels forming in the primitive nebula were selectively exposed to alien xenon from nearby supernovae or red giant stars. There are other indications that noble gases in Allende do not represent alien nucleosynthesis products. Allende's carbon contains helium and neon with normal planetary isotopic compositions²⁹, and the carbon itself has a normal value of $^{12}\text{C}/^{13}\text{C}$ (ref. 30). The $^{82}\text{Kr}/^{130}\text{Xe}$ ratio in Allende's spinels is 2.6, which is close to the value of $^{82}\text{Kr}/^{130}\text{Xe} = 1.8$ in bulk Allende²⁶ and about a factor of 10 higher than the value that Srinivasan and Anders¹⁵ report for this ratio in the gas component from red giant stars.

Variations in the abundance of the lightest and heaviest xenon isotopes in gas released by stepwise heating of Allende's acid-etched residues are shown graphically in Fig. 2. Xenon isotopes released from the carbon (C-2) and spinel/carbon (B-1) fractions define different correlation lines. These do not agree with the correlation shown by Lewis *et al.*^{26,27} for other etched residues of Allende (dashed line in Fig. 2), nor do they pass through the composition of AVCC or 'trapped' (ref. 26) xenon. Since ^{132}Xe may be produced by both r- and s-processes³¹, it is preferable to normalise the xenon spectra to a pure s-product, ^{130}Xe .

As shown in Fig. 3A, this method of normalisation permits us to compare trends in isotopic ratios with trends expected from the addition of pure s-, r- and p-nucleosynthesis products to AVCC xenon. It can be seen in Fig. 3B that xenon isotopic ratios from stepwise heating of our two Allende residues do not follow the trend reported earlier^{26,27} (dashed line in Fig. 3A), but it can be seen in Fig. 3C that ratios for the total xenon in these residues seem to do so. Isotopic ratios of the total xenon in other etched residues of Allende^{26,27} also lie along the dashed line shown in Fig. 3A, as expected by mixing only two components. But isotopic ratios of xenon released by stepwise heating of individual residues from Allende and Murchison, as shown in Fig. 3D, E and F, cannot be explained by a simple binary mixture. Kuroda³² also notes that a simple binary mixture fails to explain isotopic ratios of xenon released by stepwise heating of bulk carbonaceous chondrites. Although the spectra in Fig. 3 confirm the positive correlation between enrichments of light and heavy xenon isotopes¹, and the spectra in Fig. 1 suggest that bulk AVCC xenon may be represented as a mixture of one s-enriched component with one s-depleted component, differences in the slopes of the correlation lines in Fig. 3 indicate that the r and p products of chondritic xenon were not completely mixed before trapping. Since the r and p processes occur in the supernova explosion³¹, to explain these xenon spectra as a consequence of injections from a nearby supernova^{1,16} would require the preservation of heterogeneities within the supernova material itself as the alien xenon was transferred into the solar nebula and then trapped on a matrix of isotopically normal carbon.

Variations in the isotopic composition of krypton (Table 2) provide additional evidence for nucleogenetic effects in the residues of Allende. Since the anomalies in krypton are relatively small and others^{15,33} have recently discussed the evidence for s- and r-products in krypton extracted from acid-etched minerals of carbonaceous chondrites, a detailed discussion on the krypton anomalies shown in Table 2 is not warranted.

Nucleosynthetic products in tellurium

If Allende's carbon itself formed in the outer layers of a supernova, then other heavy elements trapped in the carbon would be

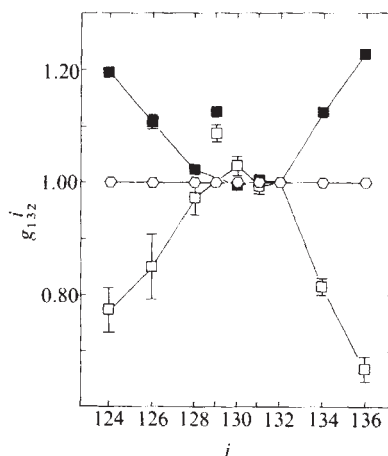


Fig. 1 Isotopic compositions of xenon in Allende's spinels ($\square$) and carbon ($\blacksquare$) normalised to AVCC xenon ($\circ$) in the manner given by equation (1). Since ^{129}Xe may contain the *in situ* decay product of ^{129}I , it is not used to define the isotopic abundance pattern of trapped xenon. Carbon is enriched in r and p products of xenon, spinels are depleted in these, and bulk AVCC xenon has an intermediate abundance pattern for the eight nonradiogenic isotopes of xenon.

expected to show enrichments of r- and p-products similar to those observed in xenon. To test this, the abundance of six stable isotopes, ^{120}Te , ^{122}Te , ^{124}Te , ^{126}Te , ^{128}Te and ^{130}Te , was determined in Allende's residues by neutron activation and γ -ray spectrometry on the (n, γ) product ^{i+1}Te . For these analyses a 32-mg aliquot of residue B, labelled B-3, was irradiated for 95 h at a flux of 6×10^{14} neutrons $\text{cm}^{-2} \text{s}^{-1}$. Monitors of tellurium, uranium, osmium and ruthenium were irradiated with the sample. Carriers of tellurium, iodine (to carry fissionogenic ^{133}I), osmium and ruthenium were added to the irradiated sample during etching to isolate the activation products from fractions of the residues rich in spinels and carbon, respectively. Standard radiochemical procedures were used to recover tellurium³⁴⁻³⁸ and iodine³⁹, and to recover and separate osmium and ruthenium^{35,40-45}. The only special reagent, potassium chloromilhexathiocyanate, was prepared as described by Morgan⁴⁵ and

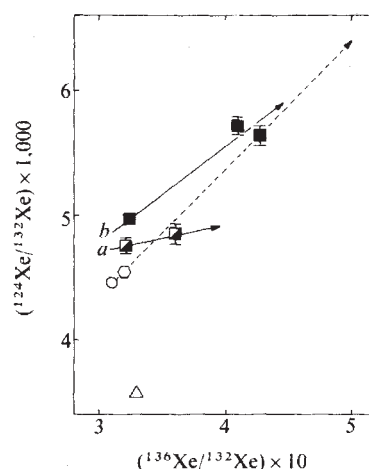


Fig. 2 Variations in abundances of the lightest and heaviest isotopes of xenon released by stepwise heating of Allende's residues. Xenon isotopes released from the mixed residue of carbon and spinels ($\blacksquare$) lie along line a, xenon isotopes from the carbon residue ($\blacksquare$) lie along line b, and xenon isotopes in other residues of Allende are reported^{26,27} to lie along the dashed line connecting 'fission-free' or 'trapped' xenon ($\circ$) and 'strange' xenon generated by fission and mass fractionation. $\circ$, AVCC; $\triangle$, air.

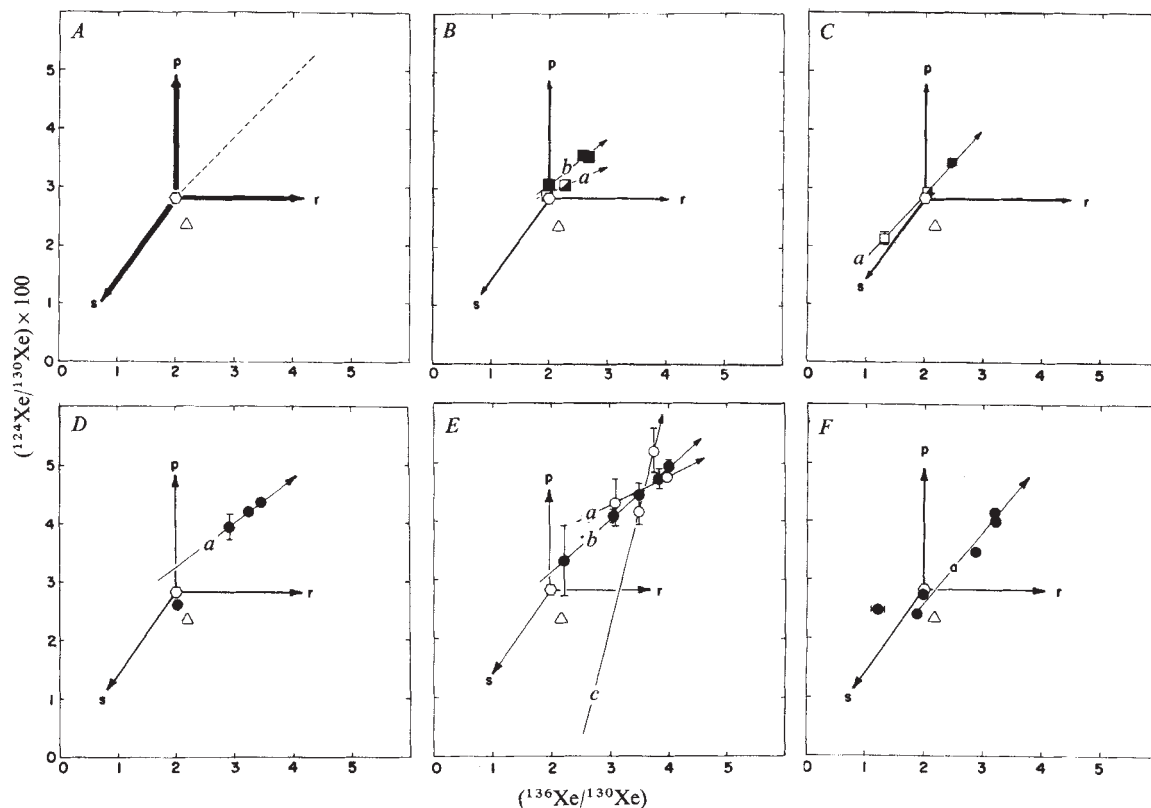


Fig. 3 Ratios among three xenon isotopes produced by the *r*, *p* and *s* processes of nucleosynthesis. *A*, A comparison of trends expected by the addition to AVCC xenon of pure nucleosynthetic components from the *r*, *p* and *s* processes (dark arrows) with trends reported from earlier analyses^{26,27} on Allende residues (dashed line); *B*, trends observed in this study for xenon released by stepwise heating of Allende's carbon (C-2) (line *b*) and carbon plus spinels (B-1) (line *a*); *C*, trends observed in this study for total xenon in Allende residues: *a* is B-1, C-2 mixing line; *D*, the trend observed in an earlier study²⁶ on xenon released by stepwise heating of one Allende residue (●), *a* is 3CS2; *E* trends observed in another study²⁷ on xenon released by stepwise heating of three Allende residues (○, ●) 4C57 (*a*), 4C56 (*b*) and 3CS59 (*c*); *F*, trends observed¹⁵ in xenon released by stepwise heating of a Murchison residue (●) (*a*, ICIO). Other symbols are defined in Figs 1 and 2. All error limits greater than the size of the symbols are shown, except for the two temperature fractions of Murchison (Fig. 3*F*) with low values of $^{124}\text{Xe}/^{130}\text{Xe}$. Error limits were not reported¹⁵ on the latter. △, Air; ◇, AVCC.

used in the precipitation of osmium and ruthenium. Attempts to measure the isotopic compositions of osmium and ruthenium were abandoned after our results showed that these elements are highly enriched on the residues, apparently as a result of their insolubility in the etching acids⁴⁶, and display no large isotopic anomalies.

A Ge(Li) detector and a thin-crystal, intrinsic Ge detector were used with a 1,024-channel analyser to determine the activity of each tellurium isotope relative to the activity of ^{129}Te in the samples and in the monitors. The latter were prepared from purified TeO_2 (lot 766061, Fisher Scientific). Possible interferences which might produce ^{129}Te from neutron-induced reactions on other elements in the samples are (1) neutron-induced fission of uranium to produce ^{131}Te and ^{129}Te , (2) the (*n*, *p*) reaction on iodine and the (*n*, α) reactions on xenon, and (3) the (*n*, γ) reaction on ^{124}Sn followed by two successive β decays. Our results indicate a ^{128}Te concentration of 16 p.p.m. in the carbon and 21 p.p.m. in spinels, corresponding to total tellurium content of 51 and 67 p.p.m., respectively, if all other isotopes were present in the proportion observed for terrestrial tellurium. The activity of fission-produced ^{133}I and ^{132}Te in each residue and in the uranium monitor indicate that interference from neutron-induced fission of uranium produces 1.1% of the ^{131}Te activity and 0.5% of the ^{129}Te activity in the irradiated carbon, and fission interference produces 0.9% of the ^{131}Te and 0.4% of ^{129}Te activities in the irradiated spinels. The results of our tellurium and xenon analyses (Table 1) yield atomic ratios of $\text{Te}/\text{Xe} \approx 5 \times 10^4$ in carbon and $\text{Te}/\text{Xe} \approx 1.5 \times 10^5$ in spinels. From the amounts of radiogenic ^{129}Xe in these residues (Table

1) and an assumed value of $^{129}\text{I}/^{127}\text{I} = 10^{-4}$ for trapped iodine⁴⁷, we calculate atomic ratios of $\text{Te}/\text{I} \approx 130$ in carbon and $\text{Te}/\text{I} \approx 480$ in the spinels. The ^{127}I (*n*, *p*) reaction and the $^{124-134}\text{Xe}$ (*n*, α) reactions have *Q* values of about 0.1 MeV and 2.5–6.3 MeV, and they are inhibited by Coulomb barriers of about 8 MeV and 14 MeV, respectively. Thus, each of the interference reactions listed under item (2) requires neutron energies above 8 MeV. All of the relevant cross-sections for (*n*, *p*) and (*n*, α) reactions are not known, but typically these are only a few millibarns for 14 MeV neutrons on target elements with $Z \approx 50$ –60 (ref. 48). In the position where our samples were irradiated, the flux of neutrons above 8 MeV is about 1×10^{-3} of the thermal flux. From the atomic ratios given above and assuming cross-sections of 1,000 mb for each (*n*, *p*) and (*n*, α) reaction, we estimate upper limits for interference on any tellurium isotope to be 0.03% from the (*n*, *p*) reaction and $1 \times 10^{-4}\%$ from the most favourable (*n*, α) reaction. Concentrations of tin, the target element for interference from above item (3), were not measured in our Allende residues. In other Allende residues prepared in a similar manner, the highest concentration of germanium, a neighbour of tin in the Group IV A elements, is reported⁴⁶ to be 37 p.p.m. If our Residue B (carbon + spinels) contained 37 p.p.m. germanium, we calculate lower limits on the atomic ratios of $\text{Te}/\text{Sn} \geq 11$ in carbon and $\text{Te}/\text{Sn} \geq 18$ in the spinels, assuming a solar value for the Sn/Ge ratio⁴⁹ and assuming that the mineral fraction being considered, carbon or spinels, each contain all of the germanium of Residue B. The ^{125}Sn produced by neutron-capture on natural ^{124}Sn must undergo two β -decays before becoming an interference at ^{125}Te . An

intermediate decay product, ^{125}Sb ($t_{1/2} = 2.73$ yr), acts to shield ^{125}Te from most of the ^{125}Sn decays, and we calculate an upper limit of 0.1% for the interference at ^{125}Te from the ^{125}Sn (n, γ, β, β) reaction. Interference from the $(n, 2n)$ reaction on ^{125}Te is expected to be negligible; for these reactions, $Q \geq 8$ MeV.

The results of counting data accumulated from 5 February 1978 to 28 May 1978 are shown in Table 3, corrected for the fission interference discussed above. It should be stressed that we measured only relative abundances of tellurium isotopes in the monitors and in the samples. The maximum variation that Smithers and Krouse⁵⁰ observed in isotope ratios in six natural tellurides and two commercial tellurium salts corresponds to a fractionation of only 0.05% per mass unit, variations which could not be detected by the techniques used in this study. We therefore assume that the tellurium in our monitors has normal values for isotope ratios, and that the activity of each isotope, ^{125}Te , is proportional to the abundance of the stable (n, γ) target isotope, Te. Thus, the activities shown in Table 3 can be expressed in an analogous manner to that given for xenon isotopes in equation (1).

$$g_{128} = (^{128}\text{Te}/^{128}\text{Te})_{\text{sample}} / (^{128}\text{Te}/^{128}\text{Te})_{\text{standard}} \quad (2)$$

The results of our analyses on tellurium isotopes in Allende residues are shown graphically in Fig. 4. As ^{126}Te may contain a radiogenic decay product from *in situ* decay of a precursor isotope produced in the *r*-process, ^{126}Sn ($t_{1/2} \approx 10^5$ yr) (ref. 51), the abundance of ^{126}Te is not used to define the general anomaly pattern of tellurium isotopes trapped in these residues. No data are available on the isotopic composition of tellurium in average carbonaceous chondrites, AVCC tellurium. Therefore, the patterns of isotopic anomalies shown in Fig. 4A (carbon) and Fig. 4B (spinel), where the isotopic ratios are normalised to the terrestrial tellurium standard, are not analogous to the patterns shown in Fig. 1 for xenon in these same residues. The xenon spectra in Fig. 1 are normalised to an AVCC xenon standard.

In spite of differences in reference standards used in Figs 1 and 4, the following observations may be significant: (1) The nonradiogenic isotopes of tellurium and xenon in Allende's carbon, as shown in Fig. 1 and Fig. 4A, produce remarkably similar anomaly patterns. Both elements are enriched in the heaviest isotopes, ^{130}Te and ^{136}Xe , and in the lightest isotopes, ^{120}Te and ^{124}Xe . These isotopes are produced on a very short time scale by the *r* and *p* processes in supernova explosions^{31,52}. (2) The spinel-rich fraction of these minerals (Figs 1 and 4B) is enriched in the intermediate mass isotopes of each element, ^{130}Te and ^{124}Xe . These isotopes are generated over long periods of stellar evolution by the *s* process^{14,31}. (3) The existence of isotopically anomalous tellurium in Allende cannot be explained by a single AVCC-type tellurium, since the isotopic spectrum of tellurium in carbon is obviously different from that in the spinels. (4) The spinel-rich fraction of Allende residues is enriched in ^{130}Te (Fig. 4B) but not in ^{136}Xe (Fig. 1). This apparent discrepancy between the abundances of *r* products in tellurium and xenon may indicate that etching the Allende residues has separated the *r* and *p* products of tellurium, just as stepwise gas extraction separated the *r* and *p* products of xenon (Fig. 3). Or this may be due to differences in the reference standards for the xenon and tellurium spectra, that is, it is possible that AVCC tellurium is systematically enriched in ^{130}Te relative to terrestrial tellurium.

Nucleogenetic heterogeneities in element abundances

In using the results of this study to evaluate the merits of models suggested for element synthesis shortly before condensation¹³⁻¹⁹, it is imperative to consider the constraints imposed by earlier analyses²⁶ on noble gases in Allende's residues. These measurements revealed elemental abundances of

Table 3 Ratios of γ -ray intensities detected from activated tellurium isotopes

Isotopes	Carbon: terrestrial	Spinel: terrestrial	Carbon: spinels	γ -Energies detected (MeV)	Half-life observed
^{121}Te	106.9 ± 2.6	102.3 ± 1.9	105.4 ± 1.4	0.573	20–30 d
^{123}Te	104.3 ± 2.5	100.1 ± 2.4	103.7 ± 2.2	0.159	120 d
^{125}Te	97.6 ± 0.5	102.4 ± 0.6	95.4 ± 0.5	0.035*	58 d
^{127}Te	105.1 ± 4.1	100.4 ± 3.9	103.4 ± 3.6	0.418	110 d
^{129}Te	≈ 100.0	≈ 100.0	≈ 100.0	0.696; 0.027*	34 d
^{131}Te	111.4 ± 4.0	117.5 ± 3.4	94.8 ± 1.7	0.775; 0.850	30 h

* Counted with intrinsic Ge detector.

helium and neon which correlate linearly with abundances of the *r* products in xenon¹⁷⁻¹⁹, as shown in Fig. 5, and the correlations yield zero values of helium and neon at $^{136}\text{Xe}/^{132}\text{Xe} = 0.32 \pm 0.01$. This value of $^{136}\text{Xe}/^{132}\text{Xe}$ contains the range of isotopic ratios for planetary xenon in air, carbonaceous chondrites and ureilites. It is now widely accepted that variations of the $^{136}\text{Xe}/^{132}\text{Xe}$ ratio in carbonaceous chondrites may be nucleogenetic^{1,5,9,12-23,33}, and that stellar evolution selectively depletes low-*Z* elements from the hotter stellar interior³¹. It can be concluded that normal, planetary xenon was synthesised in the hot stellar interior and that the inertness of noble gases made it possible for these elements to preserve a record of both

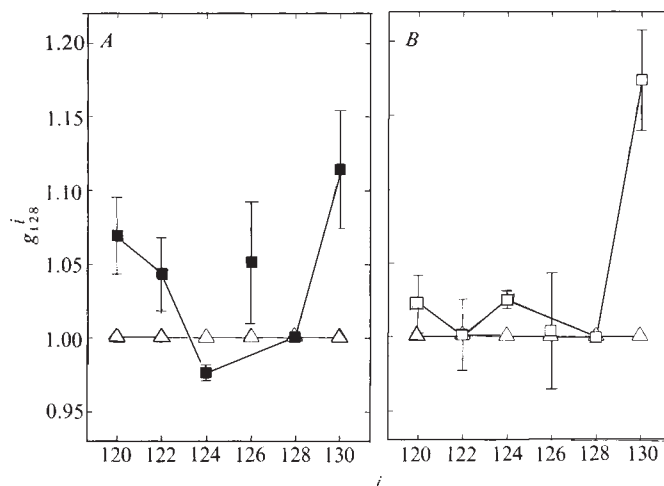


Fig. 4 Isotopic compositions of tellurium in Allende's carbon (■) and spinels (□) normalised to that in the tellurium monitor (Δ) in the manner indicated by equation (2). Since ^{126}Te may contain the *in situ* decay product of ^{126}Sn , it is not used to define the isotopic anomaly pattern of trapped tellurium. A, The anomaly pattern of tellurium isotopes in carbon resembles that observed for xenon isotopes (Fig. 1) in carbon; B, the anomaly pattern of tellurium isotopes in spinels reveals an enrichment of the *s* product, ^{124}Te , and the *r* product, ^{130}Te .

elemental and isotopic heterogeneities which existed before condensation. Most other elements formed compounds which were chemically segregated into different minerals of planetary solids. Small variations in the isotopic compositions of normal planetary xenon, as seen in bulk ureilites^{53,54}, carbonaceous chondrites⁵⁵, and air⁵⁶, are identified in Fig. 5 at values of $^{136}\text{Xe}/^{132}\text{Xe} = 0.313, 0.322$ and 0.330 , respectively. Correlation coefficients between the elemental and isotopic ratios shown in Fig. 5 are between 99.2% and 99.9%. It has been reported²⁶ that a plot of $^{124}\text{Xe}/^{130}\text{Xe}$ against $^{136}\text{Xe}/^{130}\text{Xe}$ for xenon in these same residues gave a correlation coefficient of 98%.

We did not determine helium and neon in our study but have used the trends shown in Fig. 5 in interpreting our results, since the results of other studies on noble gases in residues of Allende^{27,33,57} and Murchison⁵⁸ confirm these correlations.

These correlations are also seen in bulk noble gases of meteorites. Most carbonaceous chondrites contain helium and neon, and stepwise heating¹ or chemical etching experiments²⁶ on carbonaceous chondrites reveal the presence of an anomalous xenon component with $^{136}\text{Xe}/^{132}\text{Xe} > 0.32$. However, Marti and Wilkening⁵⁹ report that the stepwise release of gases from the Kenna ureilite revealed the presence of a single xenon component with $^{136}\text{Xe}/^{132}\text{Xe} = 0.312$, but they found no evidence of trapped helium or neon although the ureilite contained large amounts of heavy noble gases.

Conclusions

The presence of isotopically anomalous tellurium in Allende provides additional evidence for the suggestion that isotopically anomalous components in xenon in carbonaceous chondrites

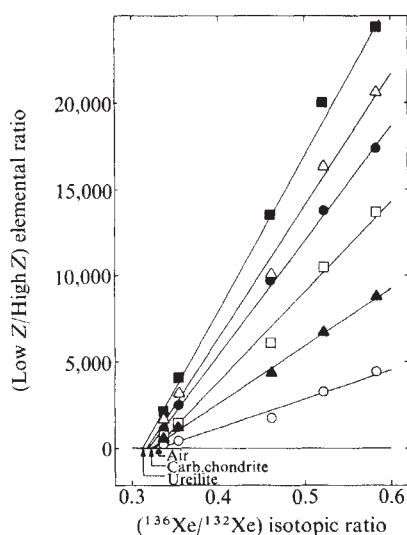


Fig. 5 Correlations between values of the isotopic ratio, $^{136}\text{Xe}/^{132}\text{Xe}$, and elemental ratios of (low- Z /high- Z) noble gases in residues of Allende²⁶. Measured values of elemental ratios are multiplied by constants so that all six ratios can be shown together. Values of $(^{20}\text{Ne}/^{132}\text{Xe}) \times 60$ are depicted by (■), $(^4\text{He}/^{132}\text{Xe}) \times 0.25$ by (Δ), $(^{20}\text{Ne}/^{36}\text{Ar}) \times 4,000$ by (●), $(^{20}\text{Ne}/^{84}\text{Kr}) \times 25$ by (□), $(^4\text{He}/^{36}\text{Ar}) \times 10$ by (▲), and $(^4\text{He}/^{84}\text{Kr}) \times 0.04$ by (○). The correlations yield zero values for the elemental abundances of helium and neon at a value of $^{136}\text{Xe}/^{132}\text{Xe} = 0.32 \pm 0.01$. This corresponds to the $^{136}\text{Xe}/^{132}\text{Xe}$ ratio of bulk xenon in air, carbonaceous chondrites, and ureilites, as shown at $^{136}\text{Xe}/^{132}\text{Xe} = 0.330, 0.322$ and 0.313 , respectively.

are nucleogenetic^{1,5,9,12-23,33} rather than fission products^{2,26,27,46,58}. The following observations are difficult to explain by alien grains carrying nucleosynthetic products from supernovae^{12,13} or red giant stars^{14,15}: (1) The carbon grains have a normal value of $^{12}\text{C}/^{13}\text{C}$ (ref. 30), (2) the carbon contains anomalous xenon, krypton and tellurium and normal helium and neon²⁹, (3) isotopic ratios of xenon enriched in s products lie on the correlation line defined by isotopic ratios of xenon enriched in r and p products (Fig. 3A, C), and (4) there was no helium or neon in the primitive noble gas component which contained normal, planetary xenon (Fig. 5 and ref. 59). Trapping of alien nucleosynthetic elements from a nearby supernova^{1,16} onto a matrix of isotopically normal material can explain observations (1) and (2), but not observations (3) and (4). The observation that r and p products from the supernova event are not mixed (Fig. 2, Fig. 3B, D–F, Fig. 4) also requires that the injected material remain heterogeneous as it was transferred from the supernova to the solar system. Even if this occurred, it seems highly unlikely that the abundance pattern of xenon isotopes from supernovae^{1,16} and red giant stars^{14,15} would be

mirror images and that these two alien forms of xenon would be fortuitously trapped in meteorites in which the bulk xenon isotopes display an intermediate abundance pattern (Fig. 1).

The isotopically anomalous components of tellurium, xenon and krypton observed in this study can be understood as remnants of the different nucleosynthesis processes which collectively produced the chemical elements of the Solar System. The opposite anomaly patterns of xenon (Fig. 1), the incompletely mixed r and p products in xenon (Fig. 2, Fig. 3) and in tellurium (Fig. 4), the association of isotopically anomalous high- Z elements with isotopically normal low- Z elements, and the absence of helium and neon in the noble gas component with normal, planetary xenon (Fig. 5, ref. 59) are consistent with the suggestion that our Solar System formed directly from the debris of a single supernova¹⁷⁻¹⁹.

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DNA sequences and structural homologies of the replication origins of lambdoid bacteriophages

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The DNA sequences for the origins of replication of the lambdoid bacteriophages $\phi 80$, 434, $\phi 21$, and λ imm21 (identical to $\phi 21$) have been determined and compared to the λ structure. Two presumptive elaborate binding sites for two initiator proteins have been identified in their outer sections, while a replicational primer start site seems to be located in their centres.

BACTERIOPHAGE λ initiates DNA replication bidirectionally from a unique site on its circularised genome. This site has been located at approximately 80% of the linear DNA molecule measured from the left end^{1,2}. A group of *cis*-dominant, replication-defective λ phages (λ *ori*⁻) has been isolated^{3,4}, and four such mutations have now been characterised by DNA sequence analysis^{5,6}. All are located within the λ *O* gene⁷, immediately to the left of an *Eco*RI restriction site at 81% λ (ref. 8). While the segment of λ DNA covered by the *ori*⁻ mutants (comprising 63 base pairs, ref. 5) gives a lower limit for the λ origin sequence, a 202 base pair upper, functional limit has been observed in hybrid plasmid cloning studies⁹.

The lambdoid phages 434, $\phi 21$, and $\phi 80$ are genetically very similar, and can exchange functionally corresponding though not necessarily identical (or heteroduplexing) segments of their genomes with λ or with each other¹⁰⁻¹². Heteroduplexing DNA segments can also be observed in electron microscope analysis of pairs of lambdoid phage DNAs¹³⁻¹⁵. In particular, the regions surrounding the origins of replication, although located at somewhat different positions within the various phage DNAs, form heteroduplexes among the subgroup λ , 434 and $\phi 21$, whereas the corresponding region of $\phi 80$ DNA seems to be different, and heteroduplexes with λ only in a right-hand section of that region. Genetic observations¹⁶ also indicate exchangeability of the analogous gene *O* products among λ , 434 and $\phi 21$, while the $\phi 80$ *O* function is not exchangeable with any of the others. The $\phi 80$ *O* protein is also different in size¹⁷. The products of the various *P* genes located to the right of the *O* genes can, however, be exchanged among all members of this group (even if only asymmetrically in one case¹⁷), although functional differences can still be detected¹⁸.

While the origin of replication constitutes at least part of the replicator structure as proposed by the replicon hypothesis¹⁹, the phage coded proteins *O* and *P* seem to fulfill the role of specific initiator proteins also predicted by this model. And the specific binding of the phage's own initiator protein(s) to its replicator structure seems to determine at least in part the individuality of these phage replicons, as being distinct from the cellular genome. In addition, besides the requirement for a supercoiled, circular DNA substrate²⁰, initiation of λ replication has been shown to depend on local transcription at or near the origin of replication (transcriptional activation)²¹⁻²⁴. When initiated, λ DNA synthesis requires most of the proteins that are essential parts of the *Escherichia coli* replication machinery, including *dnaB*, *dnaG*, and *dnaE* (polymerase III)¹⁸.

Detection (in a *colE1*- λ hybrid plasmid system) of a second, unconnected replicator element located within the *cII* gene has led us to redefine the λ (lambdoid) origin of replication as a

signal structure that constitutes (only) the start site for a replicational primer synthesis, most probably catalysed by *dnaG* coded primase⁹. DNA strand separation is a prerequisite for daughter strand DNA (or RNA) synthesis, and by definition the origin of replication in a preceding step must also be the origin of strand separation during initiation; strand separation also seems to be required for the action of primase²⁵. We assume, therefore, that facilitation of local DNA strand separation, and hence exposure of a promoter-analogous primase binding site^{26,27}, will be a basic property of the origin of replication. In addition, it may possibly also be involved in attachment of the DNA replicon to a structurally organised replication site (replisome), and in protection of the nascent replicational primer 5'-end (for discussion see ref. 28).

To look more closely at the lambdoid origin of replication we have sequenced the 434, $\phi 21$ and $\phi 80$ origin segments and compared them to λ (ref. 6). On the basis of this analysis, the λ (lambdoid) origin of replication can be deduced from structural considerations to consist of 156 base pairs, extending in both directions from its 63 base pair minimum, and included within the 202 base pair maximum size as mentioned above.

Aligning lambdoid origin DNA sequences in parallel

The various origins of replication were sequenced using the dimethylsulphate/hydrazine technique²⁹ (Figs 1 and 2). The 434 and $\phi 21$ sequences are almost identical with λ , but the $\phi 80$ DNA sequence differs from the others for most of its length. All four DNA sequences, however, are identical or at least nearly identical (see boxes in Fig. 3) in several stretches within the functional limits of the λ ($\phi 21$) origin⁹. The largest of these stretches is 31 base pairs long with only two internal $\lambda/\phi 80$ non-homologies (one is also found in $\phi 21$ DNA), and includes the *Eco*RI cleavage site common to all of them. The coding frame for this part of the *O* gene sequence also seems to be the same. With the $\phi 80$ DNA sequence clearly diverging from the other lambdoid DNA sequences to the left of position λ :1,099/ $\phi 80$:1,100 and to the right of position λ :1,169/ $\phi 80$:1,170, we regard the 31 base pair identical stretch to be a fixed point around which all the origin sequences can be aligned both structurally and functionally.

Against this background, $\lambda/\phi 80$ DNA homologies provide positive evidence for functionally important segments within the origin of replication structure, which can be cross-checked against defective λ *ori*⁻ mutant^{5,6} or other partial deletion⁹ sequences. The sequence deviations within the $\lambda/434/\phi 21$ group are considered to point to positions of minor functional importance. Although some of the sequence deviations between $\phi 80$ DNA and the other lambdoid phages may similarly point to positions of minor importance, others should indicate specific alterations to serve analogous, but phage-specific functions.

When the DNA sequences of the lambdoid origins of replication are aligned in parallel they display a surprisingly complex, but analogous order of symmetry elements. Taking other characteristic features into account, three distinct regions with very little overlap (Fig. 3) can be identified. Besides these three sections there are two short external tail segments, which differ

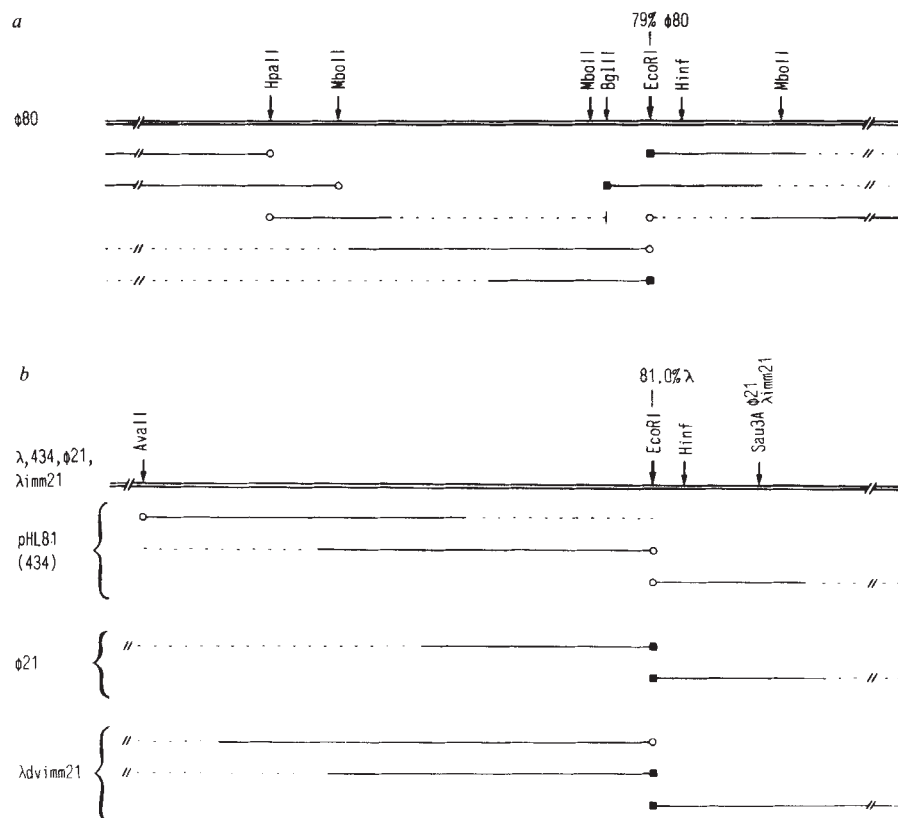


Fig. 1 Physical maps and schematic protocols for the sequence analysis of the *ori* regions of lambdoid phage DNAs. *a*, Sequence analysis of the *ori*-region of phage $\phi 80$ DNA. *b*, Sequence analysis of the *ori*-region of other lambdoid phages. Sequencing was performed with plasmid pHL81 DNA⁹, carrying the *ori*-region of phage 434, $\phi 21$ DNA, and plasmid Advimm21AB5 DNA³⁶, carrying the *ori*-region of phage λ imm21. Double-stranded DNAs are indicated by double lines, with positions of restriction endonuclease cleavage sites indicated by vertical arrows. Single lines drawn below represent single strands of restriction fragments used for sequencing, each carrying one end labelled. An open circle indicates a labelled 5'-end, and a line extending to the right of the circle, for example, represents the *l*-strand of a fragment used for sequence determination, in 5' to 3'-direction. A 3'-end labelled is marked by a square. The solid part of each line indicates the portion of the fragment for which unambiguous sequence information could be obtained. A vertical bar shows the secondary restriction site used for cleavage of one of the labelled fragments, while other secondary cleavage sites used lie outside of the region shown. Terminally labelled single strands if obtained by strand separation (for example, the *Ava*II-*Eco*RI fragment of pHL81-DNA) are not explicitly designated in the drawing.

in sequence for λ and $\phi 80$ and which do not include such structural symmetries; they probably do not belong to the origin of replication proper. An 8 out of 9 base pair identical sequence in the left tail segment may be coding for an essential element in the O protein structure, because it is read in the same frame: Ser-Glu/Ala-Trp. Only one half of an (additional) inverted repeat sequence is retained within the λ right tail sequence, the second half is located beyond the 202 base pair maximal origin boundaries—this may even be used as an argument against the remaining tail segment's being of any importance.

Conserved length of unique composition in the central region

The central (B) section (λ 1,099–1,135) of the lambdoid origins of replication consists of a stretch of DNA sequence which is uniquely rich in A in the *l*-strand, and in T in the *r*-strand, including an uninterrupted sequence of 15A+3G in the λ origin DNA, as first observed by F. Blattner and colleagues⁵. While this general characteristic is clearly seen in the $\phi 80$ origin DNA, the $\phi 80$ sequence is of slightly less extreme composition. There appear to be three segments of identical or nearly identical DNA sequences in a section B $\lambda/\phi 80$ comparison (see boxes in Fig. 3) which may be indicative of their functional importance, but the intervening groups of nucleotides are also rather similar and are identical in length, except for the deletion of one base pair in the $\phi 80$ DNA (corresponding to λ : 1,136) at the junction between the origin B and C segments.

In contrast to the flanking regions A and C, the central section of the origin of replication does not contain any inverted repeat sequences with the potential for being folded into single stranded hairpin structures; instead it very characteristically does contain DNA mirror sequences (true palindromes). One of them, in the λ -DNA group, extends over 28 base pairs with a single (λ , $\phi 21$) deviation only (considerably disrupted, however, in 434 DNA). It is immediately followed to the left by a second one of 12 base pairs (1 deviation). As a result of several nucleotide exchanges, the very long DNA mirror sequence is not

prominent in the $\phi 80$ origin of replication DNA; it is in part 'suppressed' by another, perfect 15 base pair mirror sequence, which asymmetrically overlaps with the first. Since an asymmetric DNA composition of the kind observed in section B would contribute to forming such mirror DNA sequences, they can still be maintained to be accidental, and their part in the overall origin function—if any—remains uncertain.

Segments of double-stranded DNA sequence of the composition observed for section B should have very low melting points as a result of their high A-T contents. According to reference data³⁰ the T_m can be calculated for the λ and $\phi 80$ section B DNAs to be about 78.5 °C and 79 °C, respectively (in 1×SSC; 12 °C below λ DNA average). Specialised sections of such sequence, when their strands become separated in an RNA polymerase catalysed transcriptional activation reaction (see below), should transiently be converted into a rigid, open helical conformation, due to the purine stacking interactions in their separated *l*-strands.

Specific sequences exposed in (protein coated) single-stranded templates are known to be required for binding and initiating primer RNA synthesis by *dnaG* coded primase, and the same sequences, if present in double-stranded DNA, will not be recognised by this enzyme²⁵. In a λ -colE1 hybrid plasmid genetic analysis a replicational primer synthesis has been observed to be initiated within the origin of replication boundaries in an asymmetric, only right to left orientation, and to be insensitive to an inserted transcriptional terminator⁹. λ replicational primer synthesis, therefore, seems to be catalysed not by RNA polymerase, but most probably by primase, already known to be involved in initiation of DNA replication in other systems^{23,24}. The central section of the origin, which coincidentally has a very low melting point, and contains the DNA sequences analogous to the G4 origin^{26,27}, also in a right to left orientation, seems, therefore, to be the most obvious choice for an initiation site for the λ primer synthesis observed *in vivo*. This is confirmed by the fact that the RNA polymerase is not bound to various $\phi 80$ DNA fragments carrying the origin of replication, in a filter binding assay (in 125 mM KCl, 10 mM MgCl₂,

20 mM Tris HCl (pH 8.0) at 37 °C, with competition by poly(rI) (ref. 31, results not shown). The overall rifampicin sensitivity of λ replication^{18,23,24,32} may then have to be attributed to the preceding RNA polymerase catalysed transcriptional activation of the origin structure, usually in a left to right direction across its location.

Asymmetric oligo dA:dT segments have also been observed in other, unrelated double-stranded origins of replication³³, and A-rich sequences for single-stranded phage DNA origins^{26,27}. Comparison between their sequences may lead to the identification of a promoter-equivalent primase signal structure.

Two λ origin-defective mutants, in which different parts of section B are deleted (λ r99:1,098–1,109; λ r96:1,116–1,130) have been sequenced to date⁵. Both of them show a completely defective mutant character.

Two overlapping DNA hairpin loops in the right-hand section

The origin section C shows two different characteristic organisational features which are both contained in the same stretch of the DNA. One is the exceptional, 31 base pair sequence, which is identical (with two exceptions) for all four phage DNAs. Second, this section contains two almost perfect inverted repeat sequences which overlap asymmetrically. On DNA strand separation the inverted repeat sequences could give rise to two different kinds of DNA hairpin structures (see Fig. 4). These 33 base pairs at the origin of replication (λ :1,136– ϕ 80:1,168) therefore apparently have to fulfill triple functions. First they code for 10 amino acids of the gene O product, and second and third, they act as parts of the longer and the shorter DNA hairpin loops. DNA segments with multiple functions have also in other cases been shown to be highly conserved throughout evolution³⁴.

Because the section C sequences are almost identical in all four phages, any candidate initiator protein(s) binding to them must also be identical or at least exchangeable among all four phages. These should, therefore, be the phage P proteins, or one of the host initiators, such as the *dnaB* protein (or a complex of both).

If the λ origin of replication is cut within section C at the *EcoRI* restriction site and is fused onto various foreign, *EcoRI* terminated fragments, the overall replicational activity (in the presence of the O protein) will drop to 3–5% of its original value, as observed in the λ -colE1 hybrid plasmid system⁹. This indicates that section C is an integral part of the overall λ replicator structure. The functional leakiness of the deletion variants may be due to either the activity of the remaining fraction of section C DNA, or else may indicate a potentiating rather than a basic function for section C.

DNA cloverleaf structure in the left-hand section

Section A of the lambdoid origins of replication displays the largest number of symmetry elements (Fig. 3). These are organised in the form of direct repeats, which consist internally of an inverted repeat substructure. Also, the direct repeats are very regularly spaced within the section A sequence, and therefore higher orders of symmetry involving several such elements can be considered. All such considerations of structural symmetry originate from the multiple repetitions of a basic sequence 'theme' (boxed in Fig. 3), alternating in direct and inverted orientations. While the λ and ϕ 80 linear section A DNA sequences differ from each other, they are organised in a clearly analogous manner with the total and very complex arrays of symmetry elements present in nearly identical arrangements. The sequence 'themes' basic to all of these analogous configurations, however, are different, even in length: λ , 434, ϕ 21: GAGGGAT; ϕ 80: CGGGAA.

If DNA strand separation is accepted as likely to occur at the origin of DNA replication, any one of the section A inverted sequence repetitions could be folded into a single-stranded

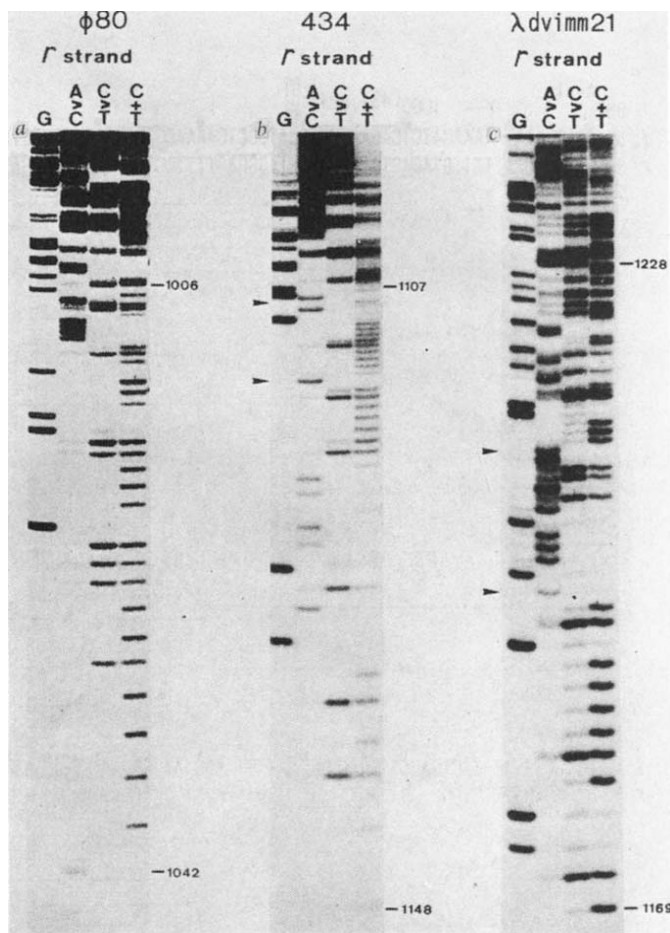


Fig. 2 Autoradiographic patterns of DNA sequencing gels after electrophoretic separation of the chemical degradation products of various origin DNA restriction fragments. The lanes designated by G, A > C, C > T, and C + T display the products of the corresponding reactions. DNA fragments derived from the various origin of replication regions as observed in preceding restriction mapping have been terminally labelled with ³²P-phosphate at either their 5'-ends by using polynucleotide kinase and [γ -³²P] ATP (ref. 29) (a–b) or at their 3'-ends by DNA polymerase I and [α -³²P] dATP (refs 37, 38) (c). For DNA sequencing the dimethylsulphate/hydrazine technique²⁹ has been used throughout this work. The numbers to the right of each gel refer to the corresponding nucleotide positions in the *l*-strand DNA sequences shown in Fig. 3, while arrows to the left (in b and c) indicate some of the sequence deviations in the *ori* regions of pHL81 (that is, phage 434) DNA (b), and λ dvimm21AB5 DNA (c), with respect to the homologous λ DNA sequence⁶. The deviations in the *ori* region of λ dvimm21AB5 DNA could also be observed in ϕ 21 DNA (compare legend to Fig. 3). a, Sequence pattern obtained from the 5'-labelled *Mbo*II fragment 338–1,046 derived from ϕ 80 DNA, which was recut by *Hae*III at position 892. b, Sequence pattern from the 5'-labelled *Eco*RI fragment of pHL81 DNA (434 DNA) 624–1,146, recut by *Bgl*II at position 792. c, Sequence pattern from the *Eco*RI-linearised, and at position 1,146 3'-labelled λ dvimm21AB5 DNA, recut by *Taq*I at position 1,466. The degradation products were loaded on 40 × 22 × 0.2 cm gels (a and b) or 90 × 16 × 0.2 cm gels (c) of 20% polyacrylamide/7 M urea, and were electrophoresed for 6 h at 1,000 V (a and b), or for 24 h at 2,000 V (c).

DNA hairpin structure, and therefore several 'activated' structural arrangements are possible for a segment of such complex organisation. A collection of potential secondary structures is represented in essence by the arrows drawn in Fig. 3. However, the only one of these potential activated structures which involves all the symmetry elements at the same time, and also makes use of their regular spacing along the DNA of this section, is a DNA cloverleaf arrangement (Fig. 5). Identical superstructures can be drawn for ϕ 80 and for λ , in spite of the differences between their individual elements. When folded into such higher order of symmetry arrays, the repeated sequences of either six or seven nucleotides form the individual branches and stems of the cloverleaf structures. Quite consistently, it is also

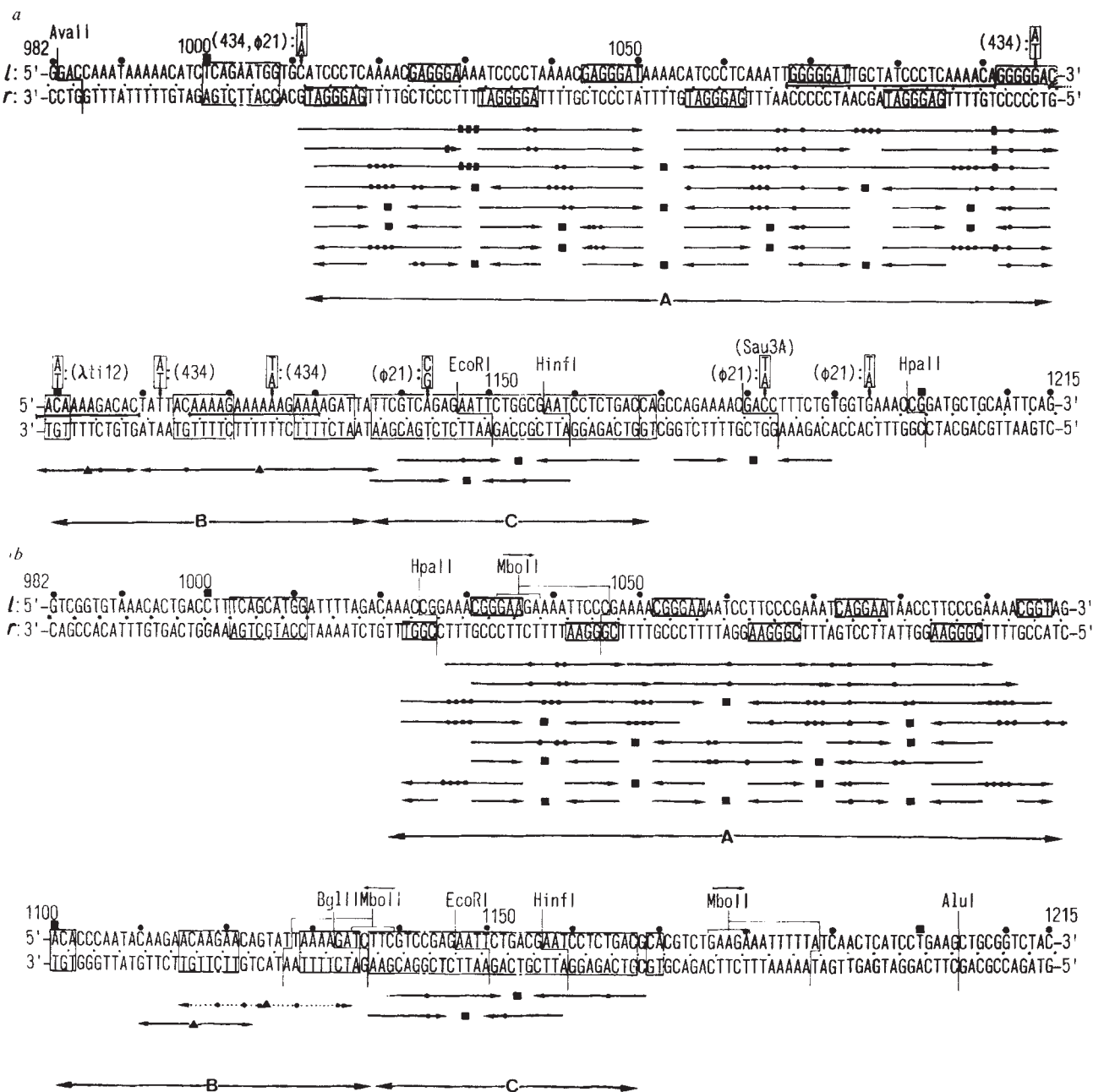


Fig. 3 DNA sequences of the λ (a) and ϕ 80 (b) origins of replication in the λ (ϕ 21) minimisation boundaries (982–1,182) (ref. 9). The nucleotide positions in all four phage DNAs are normalised, for 1,146 to indicate the first nucleotide (G) of the common *EcoRI* cleavage site located within their origin of replication sequences. This is equivalent to position 1 indicating the first nucleotide transcribed into the p_R promoted mRNA, in λ DNA³⁷. Nucleotide exchanges observed for the homologous 434 and ϕ 21 DNA in an otherwise identical sequence are indicated above the λ sequence, the position 1,100 transversion determined for the λ *i*12 mutation⁶ has also been included. Selected restriction endonuclease cleavage sites are referred to in their standard abbreviations. The *O* gene reading frames are marked by dots between the two lines showing the DNA sequences for both complementary strands, and the nucleotide segments deleted in three λ *ori*⁻ deletion mutants⁵ are also indicated (—): reading from left to right, λ r93, λ r99 and λ r96. Double strand boxes mark segments of λ / ϕ 80 DNA homology, single strand boxes point out multiply repeated sequence 'themes' present in the left sections. The symmetry elements observed in the origin DNAs are indicated below the sequences. Direct repeats have been marked by repetitive arrows, inverted repeats by two arrows pointing towards their inversion centre (square), the arrows indicating a mirror DNA sequence have been orientated away from its centre (triangle). G · T/A · C symmetry deviations in inverted repeats have been indicated by one dot (in the *l*-strand purine location). A · G/T · C mismatches by two dots (in both locations); vertical bars in a mark positions of nucleotides inserted or deleted over a perfect match. In the left parts, the lower-most arrangements of symmetry elements correspond to the activated DNA cloverleaf structures presented in Fig. 5. Three structurally different sections of the origin sequences as deduced from the distribution of symmetry elements are indicated at the bottom (A, B, C). In comparing the origin DNA sequences of λ , ϕ 21 and hybrid λ *imm*21 DNAs (plasmid λ dvimm21AB5 (ref. 36), and phage λ *imm*21 (ref. 11)) we observed that the hybrid λ *imm*21 DNA in this region consists of ϕ 21 rather than λ DNA. This has been concluded from the presence of the exchanged ϕ 21 nucleotides instead of the λ specific nucleotides at every divergent position analysed: 1,011, 1,143, 1,182, 1,194 (and 1,233; not shown). The nucleotide exchange C → T at position 1,182 results in an additional *Sau*3A recognition site not present in λ DNA, which has been used in our origin minimisation cloning studies⁹. Therefore, the origin limits as indicated are actually those of ϕ 21 DNA, which however should also apply to the nearly identical λ DNA. Restriction analysis of ϕ 21 and λ *imm*21/ λ dvimm21 DNAs in comparison with the λ DNA *O* and *P* gene sequences (ref. 6 and E. Schwarz, unpublished) resulted in recognition of a number of restriction sites present in both ϕ 21 and λ *imm*21 DNA, but not present in λ DNA, up to at least the centre of the *P* gene. Only to the right of the *P* gene is the λ *imm*21 DNA like λ DNA (not shown). λ *imm*21 and ϕ 21 DNA have also been sequenced and compared to λ DNA in the *oop* and *p₀* promoter regions⁸, and in this segment, to the right of the ' λ *imm*21 boundary' at position 595 (ref. 37) all three DNAs are identical for at least 117 base pairs. From these results we wish to conclude that the recombination event leading to λ *imm*21—(ref. 11) took place near the right rather than the left end of the *oop*-*O*-*P* homologous section of λ compared with ϕ 21 DNA. While position 595 now may have to be called a 'virtual boundary' with respect to the λ *imm*21 creation event, it can still be expected to mark the effective boundary of non-homology compared with homology in a phage λ *imm*21/ λ recombination or DNA heteroduplex analysis.

the most complex cloverleaf configuration, which results in the smallest number of hairpin mismatches (Fig. 3). This is particularly obvious for the altogether smaller (77 base pair compared with 85 base pair), and most perfect $\phi 80$ DNA cloverleaf, which in all of its three hairpin branches only contains a single A·C/G·T (*l*-strand/*r*-strand) mismatch, due to an aberrant A:T instead of G:C in position 1,074.

The potential λ , 434, and $\phi 21$ DNA cloverleaf structures have their branches and stems expanded over the $\phi 80$ DNA cloverleaf size by one hairpin base pair each. Although this should add to their stability, they do include an increased number of mismatches (three within the branches, one or two within the stem, all of the G·T/A·C type, and some other deviations concerning the left branch). Some of these aberrations may result from amino acid sequence requirements in that part of the O protein structure, for which the origin DNA sequence is coding in addition to its fulfilling DNA replicator functions.

Besides yielding nearly perfect cloverleaf branches only the origin cloverleaf model also succeeds in placing all the four regular, significantly A-rich intervening sequences into a specific, symmetrical arrangement of their own ('connecting stretches', $\phi 80$: AAAA, λ : AAAAN, with few deviations only). Stacked A₄ groups in between the cloverleaf branches are supposed to aid in lining up the protein-complexed individual cloverleaf elements for tetramerisation. The three more variant intervening groups which generally consist of A-rich, pentanucleotide sequences, through cloverleaf folding become located into symmetrical positions of their own too, and form the single-stranded top loop sequences of the individual branches. Both groups are necessary parts of the overall cloverleaf structure, in addition to the cloverleaf branches proper. Obviously, the cloverleaf stems because of their external connections have to deviate somewhat from a regular cloverleaf branch configuration. They are still close enough in their structural arrangements, however, to qualify for a fourth branch of the section A DNA cloverleaves (more fully in the λ case).

An additional point favouring the hypothesis of a higher order DNA cloverleaf structure is that it can be built from small, individual hairpin loops used as building blocks, which are then converted into a large superstructure, through a secondary, symmetrical rearrangement (of protein subunits bound to them). In our model to explain the mechanism of transcriptional activation of the origin of replication we expect RNA polymerase to act as a DNA strand separation agent which is able to catalyse the formation of such hairpin loops within the non-coding strand, while both strands during transcription are being separated internally in the enzyme molecule²⁸. Only rather short DNA hairpin loops, however, are likely to be accommodated by the enzyme molecule in such a mechanism, and activated origin structures using larger-sized individual DNA-hairpin configurations⁵ (which in the middle also would contain rather weak sections, see Fig. 3) seem to be disfavoured in this kind of an activation reaction, while a stepwise formation of individually activated cloverleaf branches would be possible.

We interpret an activated section A origin cloverleaf structure to represent the single-stranded (tetramer) DNA binding site for one of the proteins active in initiation of phage DNA replication. As judged from their analogous, but individual DNA structural designs, the initiator proteins binding to the $\phi 80$ or λ DNA cloverleaves should also be analogous, but different and non-exchangeable between the two phages (exchangeable, however, within the group of λ , 434, and $\phi 21$). The obvious choices for proteins of such specificity have to be the O proteins of all these four phages, or more precisely their N-terminal domains¹⁷. The design of the DNA cloverleaf structures seems to predict their binding of four identical O protein subunits, each of which may capture one of the RNA polymerase activated cloverleaf branches (as indicated in Fig. 5 in a rather general way). The DNA bound protein tetramer in turn is expected to aid in stabilising the overall DNA-protein conformation through protein-protein interactions. With an activation complex (sections A-C) first installed in the non-coding, A-rich *l*-strand, the comple-

mentary *r*-strand would stay single-stranded, and therefore would have a chance of also being converted into protein-complexed secondary structures, even without a special activation mechanism of its own. While a cloverleaf activated state structure of the origin section A DNA (on its own) would be clearly less stable than its double-stranded ground-state conformation, the energy to be contributed by a cloverleaf DNA-initiator protein binding reaction, in order to compensate for the formation of single-stranded sections within this structure, has been calculated to amount to not inaccessible values for the protein subunit-cloverleaf branch interaction compared with other protein-DNA binding reactions.

One of the origin defective mutants, λ r93, has been shown to delete 24 base pairs (1,068-1,091) of the section A DNA (ref. 5), which if projected upon the λ cloverleaf model would correlate with exactly its right branch and lower right connecting stretch removed. The observation of a slight degree of replicational leakiness for λ r93 (ref. 4), conceivably might not be in disagreement with a four-branch cloverleaf model, assuming a very low rate of activity for a crippled, strained three-branch cloverleaf that might be formed in that λ *ori* mutant. The

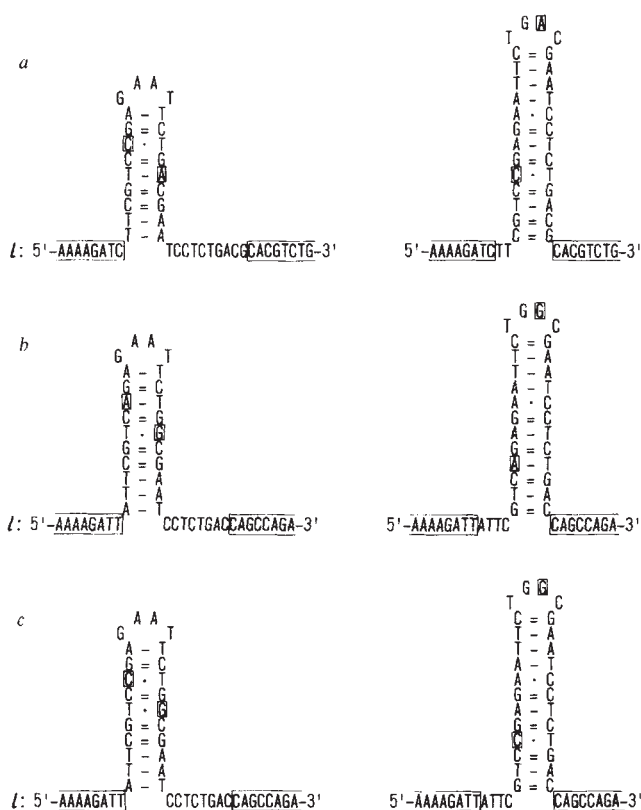


Fig. 4 Potential *l*-strand hairpin loop structures observed in the origin section C DNA sequences of $\phi 80$ (a); λ 434 (b) and $\phi 21$ (c). The smaller section C DNA hairpin loops (left) are asymmetrically overlapping with the larger section C hairpin loops (right). Left and right external boxes indicate the boundaries of section B, and right tail neighbouring DNA segments, respectively; internal boxes mark the only two positions of single nucleotide exchanges among the various phage DNAs. The left end of the smaller hairpin loop coincides exactly ($\phi 80$: 1,137) or nearly exactly (λ : 1,136; the corresponding base pair, however, is deleted in $\phi 80$) with the left end of the common, identical 31 base pair segment, while the right end of the larger hairpin loop again coincides exactly (λ : 1,167) or nearly exactly ($\phi 80$: 1,168) with the right end of the 31 base pair segment. We regard a structurally arranged potential for two rather than one, asymmetrically overlapping, both nearly perfect DNA hairpin loops (within a sequence also coding for a protein), and coincident with a stable, landmark section of general lambdoid origin homology, to be highly significant. And since both the DNA hairpin structures therefore may be of functional importance for the origin DNA C section, we are led to assume that this part of the structure may be shuttling between two different conformations of an activated DNA single-stranded state (and the ground state of double-stranded DNA structure). It has been possible to isolate an *Srl*⁰ mutation for the *EcoRI* recognition sequence at 81.0% (ref. 39); the nucleotide exchange(s) involved are, however, not yet known.

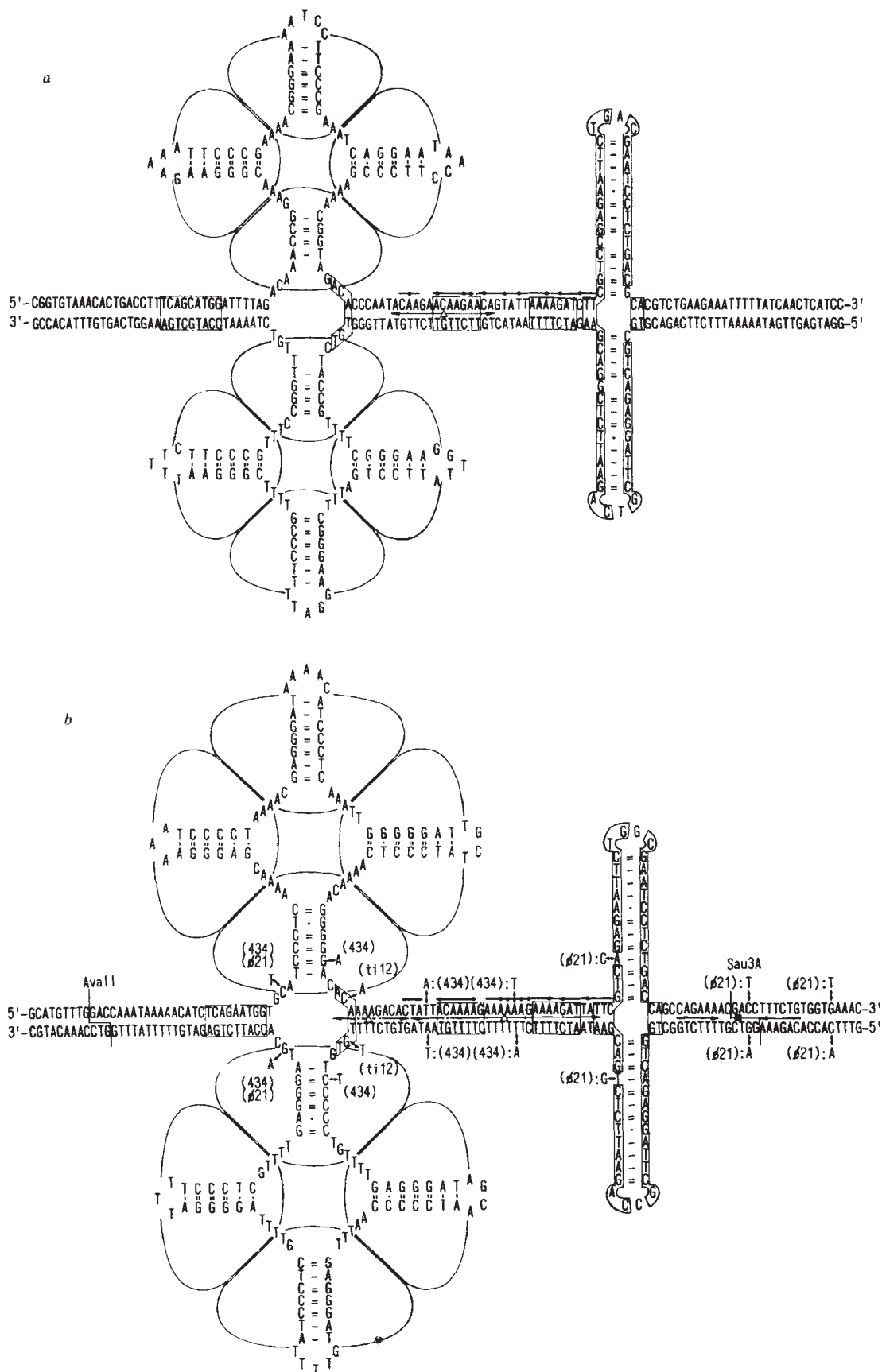


Fig. 5 Model proposed for the activated state structures of the $\phi 80$ (*a*), and λ , 434 and $\phi 21$ (*b*) origin of replication DNAs. Only the restriction cleavage sites limiting the λ ($\phi 21$) origin in its maximum functional size are indicated; for inverted repeat and DNA mirror sequence symbols see Fig. 3. Segments of $\phi 80/\lambda$ DNA homology are marked by boxes, segments of $\phi 80/G4$ and of $\lambda/G4$ DNA homologies in the central origin sections are indicated by lines above the *I*-strand sequences, with dots pointing out mismatch positions (G4: 170-172, 175-180, and 183-202 (ref. 26)). Four subunits of the (O) proteins supposed to be binding to the origin cloverleaf DNA structures have been indicated in a rather general way. Mismatches in the section A cloverleaf branches, and in the section C (larger) hairpin stems are marked by dots.

analysis of a λ r93 pseudorevertant DNA has shown the deleted section A sequence not to be re-installed³⁵, and therefore proves the λ r93 defect not to be due to the loss of the indicated segments as such, but to the disruption of an origin superstructure, of which these section A sequences are a constituent part. In replication-defective λ i12 (ref. 3) a transversion at position 1,100 (ref. 6) points out the only single nucleotide so far known to be of functional importance. Quite consistently, in constructing the two different DNA cloverleaves, the constant ACA group surrounding this position had to be placed into an identical situation with respect to both structures, as in fact both of the cloverleaves are located almost exactly in register with respect to the central sections, or to the section C DNA hairpins (36 compared with 35 base pairs for the smaller, 39 compared with 37 base pairs distance for the larger section C hairpins).

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It is certainly obvious that our model for the activated, single-stranded functional structure of the lambdoid origin of replication as deduced from DNA sequences and from genetic studies⁹, needs to be studied further biochemically, both *in vivo* and *in vitro*. However, sandwiching of a primase recognition site which is kept in a single-stranded, rigid conformation by two different initiator proteins, which in turn are complexed to two adjacent, elaborate DNA binding sites, seems to be able to explain both the basic structural as well as functional designs of such replicator signal elements.

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Domains and the hinge region of an immunoglobulin heavy chain are encoded in separate DNA segments

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A 6.8-kilobase DNA fragment containing the sequence coding for the constant region of the mouse immunoglobulin γ_1 heavy chain was cloned from total cellular DNA. Electron microscopic and nucleotide sequencing studies showed that the three protein domains and the hinge region are encoded in separate DNA segments.

SOME higher cell genes consist of informational DNA interspersed with silent sequences. Eukaryotic cistrons are often transcription units containing alternate regions to be excised from the primary transcript, introns, and to be left in the mature messenger RNA for expression, exons¹. The first indication of such a mosaic gene structure was obtained in the 28S ribosomal RNA gene of *Drosophila melanogaster*^{2,3}. This observation was followed by discoveries of an intron within the RNA leader regions of the human adenovirus late genes^{4,5}. Introns were also found in the protein-encoding regions of mouse immunoglob-

ulin λ -chain genes^{1,6}, rabbit and mouse β -globin genes^{7,8}, hen ovalbumin genes^{9,10}, and in yeast tRNA genes^{11,12}. More examples are accumulating. These studies show that introns are widespread in higher cells and their viruses, but the functions and origin of introns remain unclear.

Both light and heavy chains of immunoglobulin molecules consist of two functionally differentiated regions: the amino terminal variable (V) region and the carboxyl terminal constant (C) region. The V region recognises and binds antigens while the C region exerts various effector functions such as interaction with cell surface membrane and complement. Amino acid sequence studies have shown that both light and heavy chains consist of homology units^{13,14}. For instance, the C region of a γ -class heavy chain contains three homology regions, CH1, CH2 and CH3, each of which is similar in size (about 110 residues) and homologous to the C region of a light chain¹⁵. Furthermore, the V regions of both light and heavy chains may also be related to the C region based on the arrangement of intrachain disulphide bonds and their size, and the presence of a

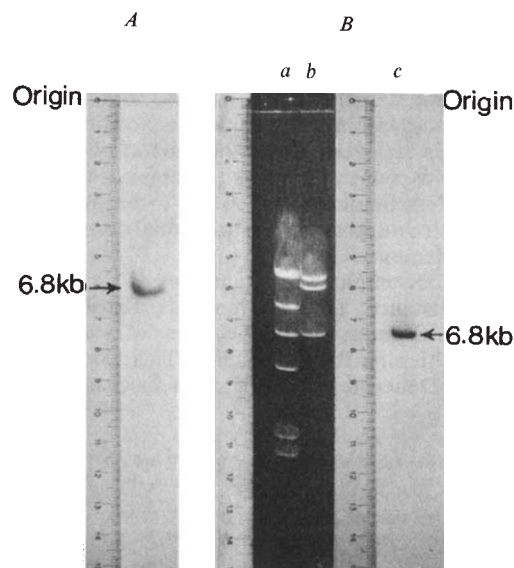


Fig. 1 Southern hybridisation of pH21-1 cDNA to myeloma MOPC 21 or cloned IgC γ_1 -1 DNA. **A**, 2 mg of high molecular weight DNA extracted from myeloma MOPC 21 was digested to completion with *Eco*R1 and separated on 0.8% horizontal agarose gel slab (20×40×1 cm) in TA buffer (40 mM Tris-HCl, 5 mM Na-acetate, 1 mM EDTA, pH 7.9) at a constant current of 50 mA for 4 days. To detect the γ_1 gene-positive DNA fragments, a 0.5-cm wide section of the gel was cut out from the centre in parallel with the direction of electrophoresis, and was blotted onto a nitrocellulose filter (Schleicher-Schuell, BA85) for 3 h as described by Southern²⁰. The filter was incubated at 65°C for 12 h in the presence of 2×10^7 c.p.m. of denatured, nick-translated pH21-1 cDNA (specific activity 2×10^8 c.p.m. per μ g) as described elsewhere¹⁹. The figure shows an autoradiogram of the filter after 6 h of exposure. The molecular size of the γ_1 gene-positive band was estimated from its relative electrophoretic mobility to *Hind*III fragments of phage λ DNA. For the cloning, DNA was recovered from the preparative slab gel as follows. Hydroxyapatite (Bio Rad, DNA grade) washed with TA buffer was mixed with an equal volume of 0.8% melted agarose (in TA buffer) and the mixture was poured into the 0.5-cm wide vertical slot in the centre of the gel slab and into a parallel slot of 0.5-cm width located near one edge of the gel. The gel slab was rotated 90° and electrophoresis was continued at 150 mA for 4 days to let DNA fragments migrate into and bind to the embedded hydroxyapatite. Hydroxyapatite containing γ_1 gene-positive DNA fragments was identified from the autoradiogram of the Southern gel blot, and a 0.5-cm thick slice of the hydroxyapatite cake in this region was cut out. The slice was suspended in 1 ml of saturated KI solution and heated at 65°C for 15 min to melt the agarose. The hydroxyapatite was collected by centrifugation and was washed with 3 ml of 10 mM KPO₄ buffer (pH 6.9). The DNA was eluted from the hydroxyapatite with 0.5 ml of 0.6 M KPO₄ (pH 6.9) buffer and the hydroxyapatite was removed by centrifugation. The DNA was dialysed against 10 mM Tris-HCl pH 7.9 containing 1 mM EDTA and was concentrated by ethanol precipitation. **B**, 0.5 μ g of cloned IgC γ_1 -1 DNA was digested with *Eco*R1, separated in a 1% agarose gel and stained with 0.5% ethidium bromide (lane *b*). DNAs were transferred onto nitrocellulose filter and hybridised with 2×10^6 c.p.m. of nick-translated pH21-1 cDNA (2×10^8 c.p.m. per μ g) (lane *c*). *Hind*III-digested phage λ DNA (0.5 μ g) was separated in the same gel as a size marker (lane *a*). The third largest *Hind*III- λ fragment is 6.6 kilobases long.

weak homology between these regions^{13,15}. These studies suggested two important points on the structure and evolution of immunoglobulin molecules. First, homology regions may have similar three-dimensional structures, each consisting of a compact globular domain. This hypothesis was directly confirmed by X-ray crystallography^{16,17}. Second, the presence of the internal homology suggested that both light and heavy chain genes evolved by duplication of a primordial gene that codes for a polypeptide chain with a size of a single homology unit¹³. Our previous studies on λ and κ light chain genes demonstrated that the V and C domains are encoded in separate exons^{6,18,19}. We here report that the three C region domains of mouse γ_1 heavy chain as well as the chain-linking hinge region between the CH1 and CH2 domains are encoded in separate DNA segments.

Identification and cloning of an *Eco*R1 fragment carrying a C γ_1 gene

High molecular weight total cellular DNA extracted from MOPC 21 myeloma was digested to completion with *Eco*R1 and the resulting DNA fragments were analysed by the Southern gel blotting technique²⁰ using as the hybridisation probe a nick-translated cDNA clone (pH21-1) prepared from MOPC 21 γ_1 chain mRNA. As shown in Fig. 1A, we detected a single band of 6.8 kilobases that hybridised with the cDNA probe. Rogers *et al.*

sequenced the γ_1 cDNA and found that it contains sequences coding for part of the C γ_1 region that extends from amino acid residue 287 to 439²¹. We conclude that the 6.8-kilobase *Eco*R1 fragment contains at least part of the C γ_1 DNA sequence.

DNA fragments in the 6.8-kilobase fraction were recovered from the preparative agarose gel and were ligated with the isolated DNA arms of an EK-2 vector, phage λ gt WES²². The recombinant DNAs were packaged *in vitro* into phage coats²³ and plaques were screened by *in situ* hybridisation using the nick-translated C γ_1 cDNA clone as probe²⁴. Screening of about 2×10^5 independently arising plaques lead to the identification of eight positive clones. When digested with *Eco*R1, DNA from the eight phage clones gave, in addition to the left and the right DNA arms of the vector, a 6.8-kilobase DNA fragment that hybridised with the C γ_1 cDNA probe. The results of one of the eight phage clones, IgC γ_1 -1, are shown in Fig. 1B.

The C γ_1 gene is split

The position of the C γ_1 -coding region in the 6.8-kilobase DNA fragment of the IgC γ_1 -1 clone was determined by electron microscopy. R-loop molecules were formed between the cloned DNA and a γ_1 chain mRNA purified from MOPC 21 myeloma cells²⁵. Figure 2 shows the various R-loop structures observed. Most of the molecules have three small R-loops, R1, R2 and R3, that are separated by two double-stranded intervening DNA segments (I1 and I2) (Fig. 2a). In all molecules a short RNA tail could be observed at the largest R-loop (R3), and in some molecules a longer RNA tail extends also from the shorter R-loop (R1).

The sites of R-loops and intervening DNA segments are summarised in Table 1. The intervening DNA segment between R2 and R3 (I2) is very short and cannot be visualised as a clear double-stranded loop, rather it appears as a little knob separating the two R-loops. The length of the two intervening DNA segments can best be measured in those hybrids where several mRNA fragments have annealed to the same DNA fragment (Fig. 2b, c). The lengths of R1 (280 base pairs) and R2 (340 base pairs) roughly correspond to the size of a DNA segment coding for a single domain (100–120 residues) of an immunoglobulin chain, while the length of R3 (420 base pairs) is slightly larger. We interpret the various parts of the observed R-loop structure in the following way. R1 and R2 correspond to the DNA segments coding for the CH1 and CH2 domains, respectively, while R3 probably encodes the CH3 domain and the presumed 3'-untranslated region of γ_1 chain mRNA. This interpretation is supported by the length and the location of the two RNA tails observed in the intact R-loop structure (Fig. 2a). The short (~200 bases) and the long (~400 bases) RNA tails can be

Table 1 Length measurements of R-loop molecules

Segment	Length (kilobases)	N
A	3.29 ± 0.13	48
B	1.92 ± 0.12	68
R1	0.28 ± 0.05	64
R2	0.34 ± 0.05	53
R3	0.42 ± 0.08	60
I1	0.51 ± 0.09	45
I2	0.18 ± 0.03	14
I1a	0.37 ± 0.06	29

The R-loops R1–R3 were measured in double-stranded molecules, in which the intervening sequence I1 was stretched out or seen as an intron-loop, and I2 was seen only in stretched out molecules (Fig. 2b). In the single-stranded DNA-mRNA hybrids the intron I1a is shortened because of the annealing of the hinge coding region. I1b is not measurable, but probably is about 100 base pairs long. The hinge region must be within 140 base pairs from the 5' end of the R2 coding region. Values are given $\pm$ s.d. N, numbers of measured molecules. Measurements of R-loop molecules were made with a Numonics digitiser and the data analysed with a Hewlett Packard HP9825 calculator.

interpreted to represent the poly A and the V-coding sequence at the 3'- and the 5'-end of the mRNA.

Correlation between exons and the domain- and hinge-coding DNA segments

In order to confirm the interpretation of the R-loop structure described above, we constructed a restriction enzyme cleavage map of the IgC γ_1 -1 clone and determined the nucleotide sequences of the relevant regions (Fig. 3*b, c*).

In determining the nucleotide sequences we first concentrated on the five regions (indicated in Fig. 3*c* as I, II, IV, V, and VI) that cover the ends of the three major exons. In so doing we discovered that coding at the right end (see Fig. 3*c* for orientation) of exon 1 and the left end of exon 2 leaves about 13 amino acid residues around the hinge region (Fig. 3*d*) unaccounted for. DNA sequencing revealed an additional exon (exon H) that codes for this cysteine-rich region within the 0.5-kilobase DNA segment separating R1 from R2. Electron micrographs of single-stranded DNA-mRNA hybrids (Fig. 2*d, e*)

are consistent with the presence of exon H. When γ_1 mRNA was hybridised to denatured IgC γ_1 -1 DNA, it annealed to the hinge region, thus forming another short intron I1b that is similar in size and appearance to I2. We thus call the four protein-encoding DNA segments exon 1, H, 2 and 3, and the three non-coding intervening DNA segments intron B, C and D as indicated in Fig. 3*c*. We also call the non-coding DNA segment to the left of exon 1 intron A because we anticipate the presence of another short exon further upstream (that is, towards the left in Fig. 3) that codes for the V-C junction region (J region)¹⁸ in analogy to the structure of a λ light chain gene.

The DNA sequences in the six regions (I-VI) indicated in Fig. 3*c* are shown in Fig. 4. Also shown in Fig. 4 are part of the amino acid sequences of MOPC 21 γ_1 chain as determined by Adetugbo²⁶, and the nucleotide sequences predicted from the amino acid sequence. Coding in exon 1 begins with Ala at residue 121 and ends in Val at residue 215. Coding in exon H starts with the Val and ends in Val at residue 228. Coding in exon 2 starts with the Val codon and ends in Gly at residue 335. Finally, coding in exon 3 starts with the Gly residue. The entire

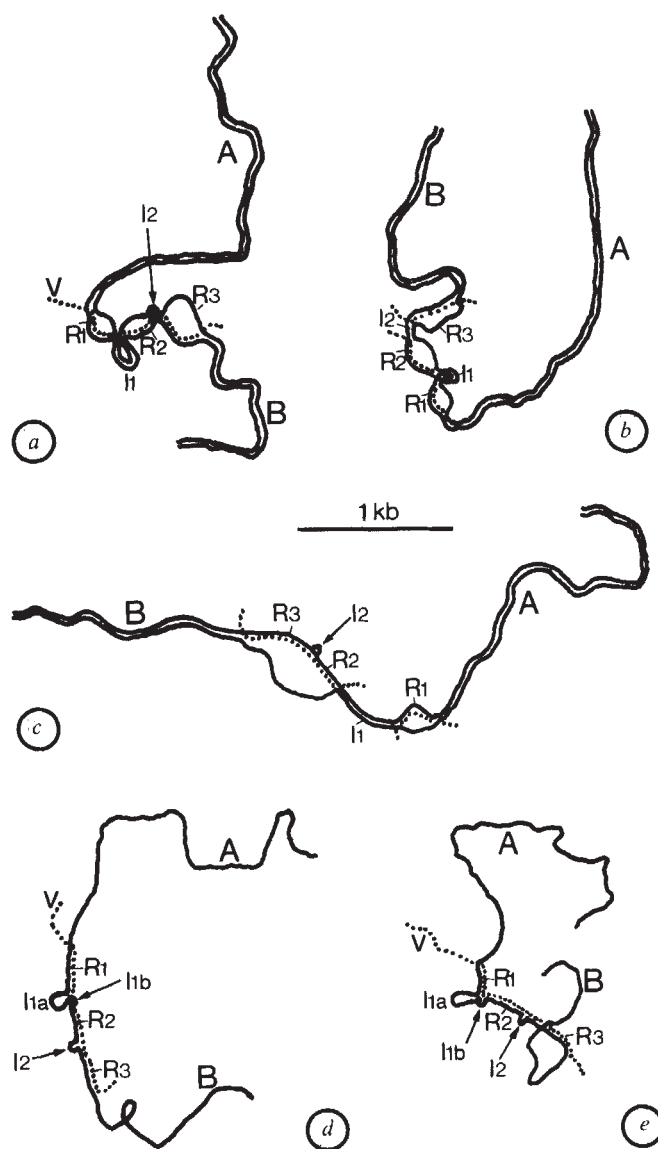
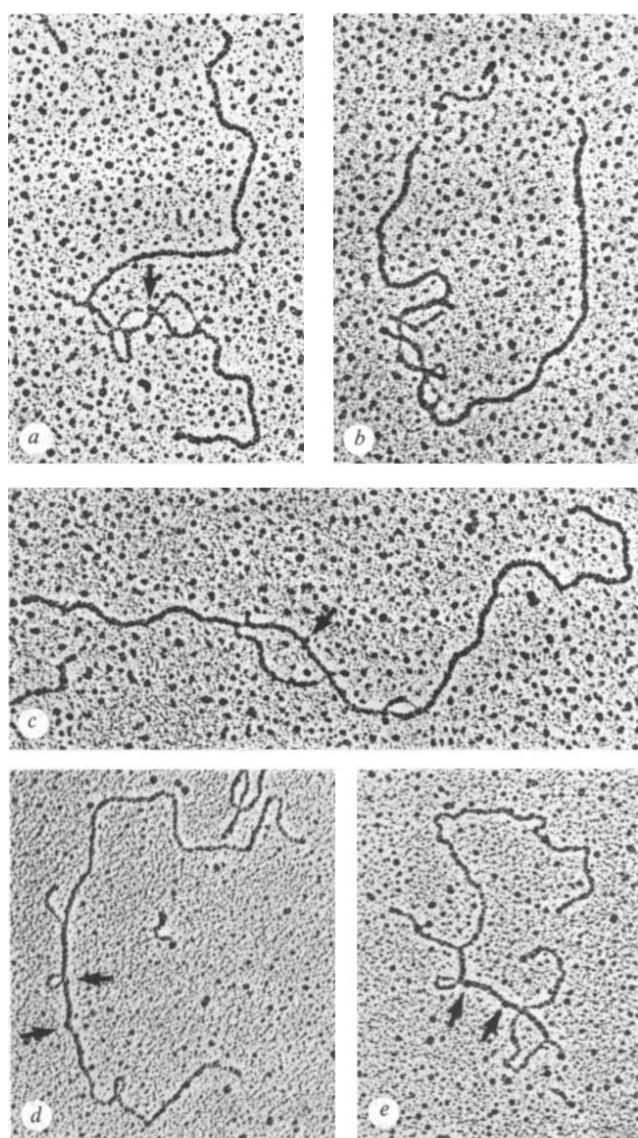


Fig. 2 Left: electron micrographs of R-loop molecules (*a-c*) and RNA-DNA hybrids (*d, e*). The *EcoRI*-digested IgC γ_1 -1 DNA was incubated with MOPC21 γ_1 mRNA under R-loop conditions at 57 °C¹⁸. Single-stranded DNA-mRNA hybrids were formed after heat denaturation and incubation of the R-loop mixture at 60 °C. The molecules were mounted for electron microscopy by the formamide-cytochrome *c* spreading method^{40,41}. Three R-loops, R1, R2, R3 are separated by two intervening sequences I1 and I2. When two separate mRNA molecules hybridise to different R-loops, the intervening sequences are stretched out and can be measured (*b, c*). In single-stranded hybrids (*d, e*) it is possible to resolve also the small intron I1b which separates the hinge coding region from the intron I1a, and which appears as a small knob beside I1a. Right: schematic interpretation of the hybrid molecules.

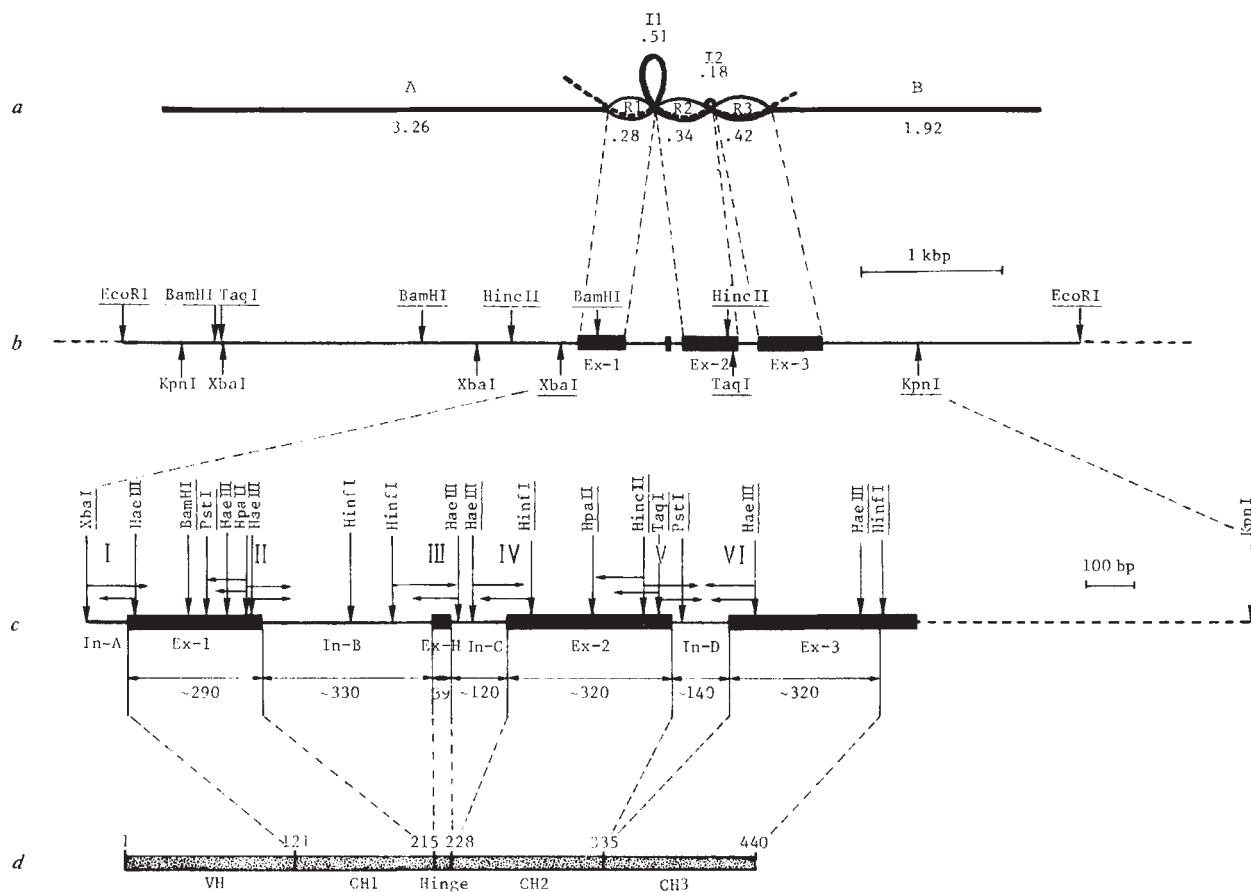


Fig. 3 *a*, R-loop map of the 6.8-kilobase insert. Heavy broken lines and heavy solid lines represent MOPC 21 γ_1 mRNA and double-stranded DNA, respectively. Duplexes between fine solid lines and broken lines are DNA-RNA hybrids. Fine solid lines are replaced single-stranded DNA. The numbers indicate lengths of various parts in kilobases. *b*, A map of the 6.8-kilobase insert obtained by the combination of restriction enzyme digestion and R-loop formation. The whole IgC γ_1 -phage DNA was digested either with one of the indicated enzymes or with that enzyme plus *EcoRI* and separated in a 1% agarose gel. Comparison of the single and the double digestion patterns allowed us to localise the outmost cleavage sites of each enzyme on the 6.8-kilobase insert. Additional cleavage sites were identified by comparison of the electrophoresis patterns obtained after digestion of the isolated 6.8-kilobase insert with one enzyme or various combinations of two enzymes. To correlate the restriction map with the R-loop map, we analysed R-loops formed between the γ_1 mRNA and the insert which had been digested with respective enzymes. The cleavage sites identified this way are underlined. R1, R2 and R3 in (*a*) correspond to Ex1, Ex2 and Ex3 in (*b*) respectively. *c*, Detailed restriction enzyme map around the exons. Sites for *HaeIII* and *HinfI* were mapped in the five isolated pieces generated by *Taq* and *Bam* cleavage of the 6.8-kilobase insert. For each piece in turn, parallel digests were done with *HaeIII* and *HinfI*, and the products were electrophoresed on 6% polyacrylamide. They were compared with similar digests of the whole insert. By noting the size of the fragments which were present only in the digests of the whole insert, and of the fragments generated from them in the *Bam*- and *Taq*-cut material, most of the sites around the *Bam* and *Taq* sites could be located. Within and between exon 1 and exon 2 all the remaining sites could also be identified, since there was only one way in which the fragments observed on single and double digests could be self-consistently ordered. Two small (30–40 base pairs) *HaeIII* fragments had to be postulated to make up the total length of this region. One of these was confirmed, and some remaining ambiguities were resolved by labelling the 5' ends of *Bam*-cut pieces of the 6.8-kilobase insert with [γ - 32 P]ATP, cutting with *HinfI*, isolating the single end-labelled fragments by preparative polyacrylamide gel electrophoresis, and analysing polyacrylamide gel electrophoresis, and analysing partial digests of them by *HaeIII*. Within exon 3, the 217-base pair *HaeIII* fragment was identified by comparison with a *HaeIII* digest of pH21-1, the cDNA clone, in which the same fragment is present. The underlined cleavage sites were confirmed by Southern gel blotting analysis using the pH21-1 cDNA probe. The lengths (in base pairs) of introns and exons are based on the DNA sequencing data as well as on the size estimation of restriction fragments. Hinge coding region (exon H) was located between exon 1 and exon 2 by DNA sequencing (see text). The roman letters indicate six regions that were sequenced. Horizontal arrows indicate actual fragments used for sequencing. In, intron; Ex, exon. *d*, Correlation of domains and hinge of MOPC 21 γ_1 heavy chain to four exons of C γ_1 -1 DNA. Numbers represent the amino acid positions at the domain-hinge or domain-domain boundaries. Numbering starts with the first NH $_2$ -terminal asparagine of the γ_1 chain.

amino acid sequence of the MOPC 21 γ_1 chain was determined by Adetugbo²⁶. While the presence of the sequence homology among the three C region domains is obvious, the exact domain boundaries are ambiguous. Nevertheless, examination of the internal sequence homology in the γ_1 chain and comparison of its sequence with that of a human γ_1 chain¹⁵, for which the three dimensional structure of the Fab fragment is available^{16,17} makes it possible to draw every domain boundary within short regions that contain no more than a few amino acid residues. Thus CH1-hinge, hinge-CH2 and CH2-CH3 boundaries can be placed around residues 214–218, residues 227–228, and residues 332–335, respectively. Furthermore, the amino terminal end of the CH1 domain is around residues 115–122. The nucleotide sequences show that in all cases coding ends or begins with a residue that lies within one of these inter-domain regions deduced from structural analysis of the protein. Since it is extremely unlikely that such a correlation is a coincidence, we

conclude that each of the three C γ_1 domains and the hinge region are encoded by separate exons. Indeed one may look at it in the opposite sense and say that the exact ends of domains and the hinge region are defined by the intron-exon boundaries on the DNA.

In the coding regions the fit between the determined and the predicted nucleotide sequences is good. However, note the discrepancies in 10 codons in the regions shown in Fig. 4. Of the 10 differences, 9 are due to exchange or permutation of residues at nearby positions (for instance the Pro-Ser exchange in exon 1) and the tenth is between Ser and Thr. Thus these discrepancies are probably due to errors in determination of the amino acid sequence. Arg-Lys exchange in exon 3 was found previously²¹ in the sequence of cDNA clone, pH21-1.

Are there any additional introns within the DNA segments coding for the three domains? The entire sequences of exon 1 and exon 2 segments have been determined (K. H. and H. S.,

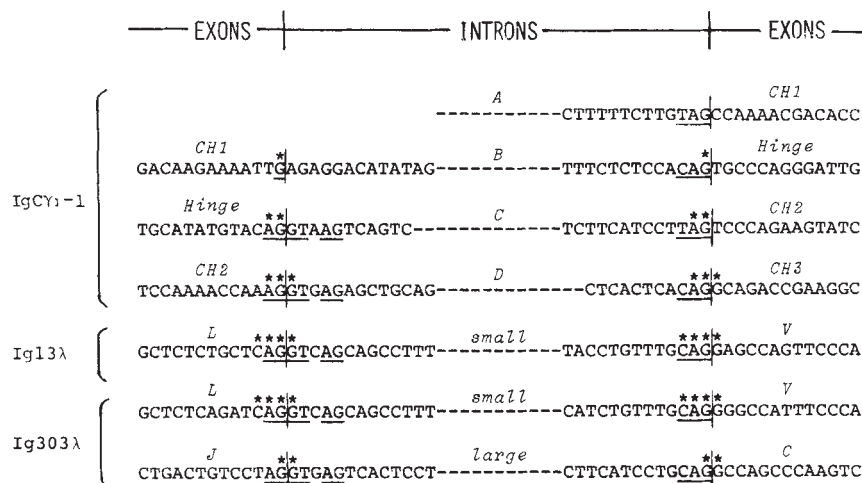


Fig. 5 Sequences around the splice sites of three immunoglobulin genes. Nucleotide sequences around the splice sites of the IgC γ_1 -1 clone as well as those of Ig13 λ ¹ (contains an embryonic V λ_{II} gene) and Ig303 λ ³⁰ (contains a myeloma (V+C) λ_1 gene) are summarised. Intron-exon boundaries defined by the GT-AG rule³¹ are indicated by vertical lines. The short 'common' sequences near the 5'- or 3'-end of introns are underlined. In case of intron B which does not follow the GT-AG rule, the boundaries indicated are based on the assumption that the intron ends with AG (see text). Nucleotides repeated near the opposite ends of an intron are marked with asterisks. CH1, CH2 and CH3 designate the three constant region domains of the γ_1 heavy chain. L, V, J, and C designate the hydrophobic leader, variable, joint, and constant region of the λ_1 or λ_{II} light chain.

which an entire domain or the hinge region is deleted. Franklin and Frangioni reported several examples of so-called human heavy chain disease proteins with such characteristics³⁵. In the present context, one mouse γ_1 chain mutant isolated from cultured MOPC 21 myeloma cells is of particular interest³⁶. This mutant, called IF2, lacks the CH1 domain extending from residue 121 to residue 214. The deleted amino acids are exactly those that are encoded by exon 1 (Fig. 4). The so-called C κ fragment found in myeloma MPC 11 may be another example³⁷. The sequence of the amino terminal region of the C κ fragment that had been synthesised in a cell-free translation system under the direction of purified C κ fragment mRNA has been determined^{38,39}. The sequence suggests that the C κ fragment is synthesised in the cells as a precursor carrying a hydrophobic leader peptide at the amino terminus. In the precursor a 17-residue long leader is directly attached to the C κ region peptide. Our previous results showed that both the leader and the V regions are encoded in separate exons in mouse λ chain genes^{1,30}. If the same applies to κ chain genes, the C κ fragment precursor may be considered as a deletion variant in which the entire peptide encoded in the V-coding exon is deleted.

Assuming that the splicing enzyme can potentially bridge two non-adjacent exons, then all deletions which begin in one intron and end in another could generate a single mutant protein in which the entire polypeptides encoded in exons between the two introns are deleted. Alternatively, a mutation at or near a splice site, whether it is a deletion or a base change, could perturb the splicing pattern such that the entire sequence in one exon is skipped in the mRNA. For instance, certain mutations near the intron B-exon H boundary may block splicing of exon 1 with exon H and in turn may promote its splicing with exon 2. Cloning and analysis of the relevant DNA fragments from mutant cells will test these models directly.

Evolution of immunoglobulin genes and origin of introns

How splicing arose in evolution is unknown. Crick has suggested that splicing was originally a protection mechanism, evolved by the host organism to reduce the damage being inflicted by the insertion of nonsense sequences in the middle of the gene (F. Crick, personal communication). In this hypothesis, the introns are viewed as DNA elements that were once inserted more or less randomly within a pre-existing gene. The placement of introns in ovalbumin and β -globin genes indeed seems to be random. However, the presence of introns at the regularly spaced boundaries of domain-coding DNA segments and at the boundaries of the domain- and hinge-encoding DNA segment is in apparent contrast to such a 'random' insertion of DNA elements. To explain the regularity observed in the location of the introns we propose the following scheme as a possible and

likely process of the evolution of an immunoglobulin heavy chain gene. It is likely that when the putative primordial gene duplicated, the unit of duplication was substantially larger than the unit of translation. As DNA duplication is likely to involve some form of illegitimate recombination which makes use of accidental short sequence homology, there is no *a priori* reason why a domain coding DNA segment would be duplicated immediately adjacent to itself. Most probably, the duplicated DNA would contain a spacer between the two domain-encoding DNA segments. One obvious way to fuse the DNA segments is to delete the spacer. But, for exactly the same reason as given above for the duplication step, such a deletion is probably a rare event. Instead, the organism might very well have resorted to a pre-existing splicing mechanism that might have developed under an evolutionary pressure such as that suggested by Crick.

How did the necessary splice sites arise at or near the ends of the domain-encoding DNA segments? One possibility is the primordial domain gene was itself an intact exon bound by splice sites. Alternatively, splice sites might have been created *de novo* by drift at the proper regions after duplication. In most cases, there seems to be a basic sequence of four to six bases to which sequences near the margins of introns are related in varying degrees. While it may not be difficult to imagine that a proper sequence of this length is generated rather frequently by drift, the specificity provided by these sequences cannot be sufficient. The same sequence is also found within exons³⁴. The obvious possibility is that combination of some higher order structure and the sequence information determine the specificity.

To generate the four-domain heavy chain gene the process outlined above is imagined to have been repeated. An additional aspect of the evolution of heavy chain genes is the generation of the hinge-coding exon. While it is possible to imagine that this exon arose from part of the ancestral intron between exon 1 and exon 2, there are two more interesting possibilities. One is that the hinge evolved by reduction of an extra C region domain corresponding to the CH2 domain which is found in place of hinge in μ and ϵ chains⁴⁴. The other is the DNA segment containing the hinge-coding sequence with or without ready-made splice sites at its margins was inserted into the ancestral intron between the exon 1 and exon 2.

Whichever variation of the model described above is more likely to be correct, it suggests that the splicing mechanism, with or without recombination at the DNA level, enables an organism to create a new gene by assembling DNA pieces each coding for a polypeptide chain of some functional use¹. If such a gene creation process indeed took place in evolution, some of the present day introns should be regarded as spacers between assembled exons and not as insertion sequences *per se*. As suggested by the hinge-coding exon and the hydrophobic leader-coding exon^{1,30}, the correlation between a clearly defined protein domain and an exon is not a necessary element of the

proposed gene creation process. A DNA segment that codes for a protein of some function and flanked by splice sites, whether they arose from the margins of insertion elements or by mutational drift, can combine with another such segment coding for a protein of a similar or an entirely different function. The function could be for binding with a specific ligand, for providing disulphide bridges or even for simply providing a peptide that can stabilise the overall structure of the newly created protein.

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Photon decay in curved space-time

RECENT astrophysical observations^{1,2} have raised doubts as to whether the large redshifts of distant galaxies (the Hubble redshift) are due entirely to cosmological expansion. The strongest argument³ in favour of cosmological expansion is that there is no known hypothesis consistent with the laws of physics (other than the Doppler shift hypothesis) that can explain the observed redshifts. An alternative explanation—a gradual energy loss of photons due to their interaction with curved space-time—is considered here. The basic premise is that because photons have a finite spread they are subject to tidal stresses and that this provides a mechanism for the transfer of momentum from the photon to the mass producing curved space-time. Any transfer of momentum without an equivalent transfer of energy will destroy the concept of the photon as a single elementary particle. It is therefore postulated that the interaction of the photon with curved space-time causes it to lose energy in the form of very low energy secondary photons. As well as providing an explanation for the Hubble redshift this hypothesis can also explain the solar limb effect, that is, the increasing redshift of solar spectral lines as the viewpoint approaches the limb of the Sun.

Most texts that consider the passage of light in curved space-time equate the trajectory of a photon to that of a light ray, thereby neglecting any lateral spread the photon may have. If we exclude the possibility of infinite energy density then in some sense not yet defined a photon must have a finite non-zero cross-sectional area. A better model for the photon is a bundle of light rays. The effect of the tidal stresses on this bundle is shown by their relative deviations from a central reference geodesic. An appropriate coordinate system is an orthonormal

Riemann coordinate system that is parallel transported along this central geodesic. Furthermore, every point along the central geodesic is uniquely labelled by the value of an affine parameter τ . As the deviations that are linear in τ given only reversible focusing (or defocusing) effects they will be ignored and we shall consider only the irreversible deviations that are quadratic in τ . These are given by the familiar equation for geodesic deviation.

The propagation of light in curved space-time is identical to propagation in a medium with a heterogeneous refractive index⁴. As the solution of Maxwell's equations is much easier for the latter case this approach is adopted here. The refractive index that gives the same geometric behaviour of light rays as the deviation of geodesics is radially symmetric about the axis of propagation and has the form

$$n = (1 - \varepsilon^2((x^2)^2 + (x^3)^2))^{1/2} \quad (1)$$

where ε is a small constant and the central geodesic is along the x^1 axis. Then to order ε propagating wave solutions can be found with a gaussian amplitude distribution in the radial direction and requiring the dispersion relationship

$$\omega_0^2 = c^2 k_0^2 + 2\varepsilon\omega_0 c \quad (2)$$

where ω_0 is the circular frequency and k_0 is the wavenumber. This gives a group velocity of $v = (1 - \varepsilon/k_0)c$, where c is the velocity of light along the reference geodesic.

Equation (2) can be interpreted as giving the wave packet an effective mass of $m = (2\varepsilon/k_0)^{1/2}(E_0/c^2)$ where $E_0 = \hbar\omega_0$ is the energy of the wave packet. The basic hypothesis here is that the same result applies to a photon in curved space-time and that this effective rest mass is just the energy of two secondary photons that are being emitted. Two secondaries are required to preserve spin. Each secondary has a wavenumber $k = (\frac{1}{2}\varepsilon k_0)^{1/2}$.

The Heisenberg uncertainty principle ($\Delta k_0 \Delta x \approx 1$) can be applied to one of the secondary photons to estimate the rate of production of these secondaries and hence the rate of energy loss by the primary photon. Then with $\Delta k_0 = k = (\frac{1}{2}\epsilon k_0)^{1/2}$ the distance between secondary emissions is given by $\Delta x = (\frac{1}{2}\epsilon k_0)^{-1/2}$. Then the energy loss rate is equal to $2\hbar \Delta k_0 / \Delta x$, which is

$$\frac{dE_0}{dx} = -\epsilon E_0 \quad (3)$$

Finally, it is possible to find the correspondence between the parameter ϵ in the refractive index equation and the equation of geodesic deviation. For a light ray travelling along the x -axis in an inhomogeneous medium there is the general result that $\frac{d^2 y}{dx^2} = -\frac{1}{n} \frac{dn}{dy}$. Combining this with equation (1) and the equation for geodesic deviation⁵ we find

$$\epsilon^2 = -(R_{\beta 2\delta}^2 + R_{\beta 3\delta}^2) \frac{dx^\beta}{d\tau} \frac{dx^\delta}{d\tau} \quad (4)$$

with $\beta, \delta = 0, 1, 2, 3$ where $R_{\beta\gamma\delta}^\alpha$ is the Riemann-Christoffel curvature tensor, and the distance is now measured by the more general affine parameter τ .

Evaluation of ϵ is readily done in the Post-newtonian approximation to general relativity. In this approximation the metric tensor is diagonal with elements

$$g_{00} = -(1 + 2\phi + 2\phi^2) \\ g_{ii} = 1 - 2\phi \quad (i = 1, 2, 3)$$

where ϕ is the newtonian gravitational potential. The result is

$$\epsilon^2 = 2\nabla^2 \phi - 2\phi \frac{\partial^2 \phi}{(\partial x^1)^2} + 2 \sum_{i=1}^3 \left(\frac{\partial \phi}{\partial x^i} \right)^2 \quad (5)$$

with the coordinate x^1 being used as the affine parameter. This gives ϵ the dimensions of inverse length. For a system of uniform density ρ we can use Poisson's equation to get from equations (3) and (5)

$$\frac{1}{E_0} \frac{dE_0}{dx} = -\left(\frac{8\pi G\rho}{c^2} \right)^{1/2} \quad (6)$$

where the replacement $x^1 \rightarrow x$ has been made. Near a single mass like the sun we have $\phi = -\frac{GM}{c^2 r}$ giving

$$\frac{1}{E_0} \frac{dE}{dx} = -\frac{2GM}{c^2 r^2} \left(1 - \frac{3x^2}{2r^2} \right)^{1/2} \quad (7)$$

Note that equation (6) comes from the first term in equation (5) which is linear in ϕ whereas equation (7) comes from the other terms that are quadratic in ϕ .

As the energy loss rate predicted by equation (6) is proportional to the photon energy the observed frequency shift cannot be distinguished from a doppler shift. It is of interest to determine whether equation (6) with the density of the intergalactic medium can account for the Hubble redshift in a static universe. For a density of n hydrogen atoms m^{-3} the predicted redshift is

$$z = (\lambda - \lambda_0)/\lambda_0 = e^{-Hx} - 1 \approx Hx \quad (8)$$

where $H = 5.59 \times 10^{-27} n^{1/2} m^{-1}$. In more familiar units this is $51.7 n^{1/2} \text{ km s}^{-1} (\text{Mpc})^{-1}$. Comparison with the conventional value⁶ of $55 \text{ km s}^{-1} (\text{Mpc})^{-1}$ shows that this theory can explain the Hubble redshift if $n \approx 1 m^{-3}$.

The most direct measurement of the density of the intergalactic plasma has been made by Field and Perrenod⁷ who find that the background X-ray observations come from a fully ionised plasma at a temperature of 10^8 – 10^9 K and a density of $n = 1.6 C^{-1/2} m^{-3}$, where $C = \langle n^2 \rangle / \langle n \rangle^2$ is a clumping factor. However, this result was obtained with an expanding universe, where the density at past epochs was greater than this value. For a static universe of constant density (but still allowing an energy-loss with distance) a reanalysis of the same data gives $T =$

1.3×10^9 K and $n = 2.4 C^{-4/3} m^{-3}$, which gives a Hubble value of $80 C^{-2/3} \text{ km s}^{-1} (\text{Mpc})^{-1}$. Unfortunately there is no direct evidence as to the value of the clumping factor C that would give a direct estimate of the Hubble constant. For this density the conventional value of $55 \text{ km s}^{-1} (\text{Mpc})^{-1}$ requires that $C = 1.8$ and that there is an upper limit to the Hubble constant of $80 \text{ km s}^{-1} (\text{Mpc})^{-1}$.

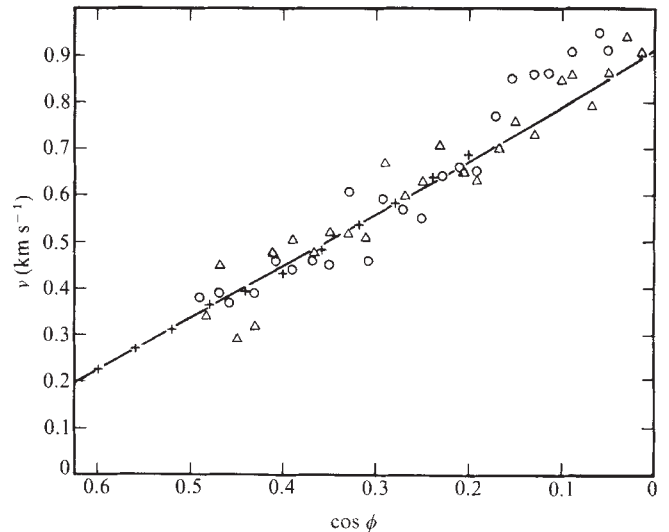


Fig. 1 The predicted (solid line) and observed solar limb effect. The redshift, expressed as a velocity is plotted against $\cos(\phi)$ where ϕ is the angle between the line of sight and the solar radius. The solid line is the predicted shift from equation 9. The experimental results are: + Plaskett¹⁰; Δ , Adam⁸; and $\circ$, Higgs⁹. All experimental results have been corrected for an apparent radial velocity of 0.39 km s^{-1} (ref. 11).

As this is only a plausibility argument several important corrections have been omitted. The first is that the cosmological curvature has been ignored. This could increase H by $\sim 15\%$. The second is that relativistic corrections to the Gaunt factor (used in calculating the X-ray emission intensity) have been omitted. Finally the assumption of an iso-thermal intergalactic plasma has been made in spite of possible density variations. Nevertheless, I suggest that the Hubble redshift could be the observed result of a photon-gravitational interaction.

Careful measurements of the Fraunhofer lines wavelengths show a small but systematic redshift across the solar disk reaching a maximum on the limb. The effect can test the validity of the second order terms in the energy loss as given by equation 7. If ϕ is the angle between the line of sight and the solar radius, the expected redshift (expressed as an equivalent doppler velocity) is given by

$$v(\phi) = \frac{2GM}{Rc \sin(\phi)} \int_{\phi_0}^{\phi} (1 - \frac{3}{2} \cos^2(\phi))^{1/2} d\phi \quad (9)$$

where $\cos^2(\phi_0) = 2/3$. The effect is zero for $\phi < \phi_0$ and then increases monotonically to a value of 0.911 km s^{-1} at the limb. Equation (9) is plotted in Fig. 1 together with the observations of Adam⁸, Higgs⁹ and Plaskett¹⁰. All the observations have been corrected for a radial velocity of 0.39 km s^{-1} (as determined by Plaskett¹¹). There is excellent agreement with the r.m.s. residuals from Plaskett's data being 0.012 km s^{-1} and for each of the others 0.05 km s^{-1} .

Integrated over the whole disk the average equivalent redshift velocity is 0.191 km s^{-1} in addition to the normal Einstein gravitational value of 0.634 km s^{-1} . Limb darkening alters this to 0.153 km s^{-1} (from Allen¹², with $u_2 = 0.84$, $v_2 = -0.20$). For other stars with similar limb darkening the equivalent redshift velocity is $0.787 m/r \text{ km s}^{-1}$ where m and r are measured in solar units. This value is not inconsistent with observations of white dwarfs and may help to resolve some disagreements between gravitational radii and those estimated from model atmospheres.

This theory clearly predicts that if the line of sight to a radio source or a spacecraft passes near the Sun there will be a substantial redshift in the observed frequency. Observations¹³ of Taurus A at 1,420 MHz with an impact parameter of $5R_{\odot}$ suggest a possible shift of ~ 120 Hz, whereas observations¹⁴ of W28 at 1,612 MHz showed no shift. The most precise results^{15,16} are observations of the Pioneer-6 spacecraft at 2,292 MHz. A consistent and unexplained redshift was observed as the spacecraft went behind the Sun, being 120 Hz at $4R_{\odot}$. The anomaly is that this theory predicts a shifts of 3,500 Hz in this case and shifts of $\sim 2,000$ Hz for the radio sources.

The explanation of the discrepancy possibly lies in an inhibition effect due to the presence of the solar corona. Efficient emission of radiation requires phase coherence of the emitting mechanism over a distance of the same size as the emitted wavelength. In this case the distance required is of order $k^{-1} = (\frac{1}{2}\epsilon k_0)^{-1/2}$. It is convenient to think of the secondary radiation being produced by an interaction between the primary photon and a gravitational wave. However, the presence of a plasma, in this case the solar corona, alters the velocity of the photon but not that of the gravitational wave. If the primary photon has a frequency low enough to suffer a relative phase shift of π radians over the emission distance the rate of secondary emission will be decreased substantially. For a plasma this critical frequency, ω_c , is given by $\omega_c = (2\omega_p^4/\epsilon c)^{1/3}$ where ω_p is the circular plasma frequency. Typical values for the solar corona are $\omega_c = 1.0 \times 10^{11}$ Hz at $3R_{\odot}$ and 2.3×10^{10} Hz at $10R_{\odot}$, showing that the critical frequency is well above the observational frequencies; thus strong inhibition of the interaction seems likely.

This is not the first proposal that relates the redshift to the effect of mass or gravitational fields on photons. Furth¹⁷ derived an expression for frequency shift proportional to curvature by analogy with an electron in a circular orbit. However, he considered curvature in three spatial dimensions only and suggested that the energy loss went into gravitational waves. Stephenson¹⁸ proposed that there would be a redshift due to absorption or shielding of gravitational flux. Gillieson¹⁹ used a mechanical model for the photon to explain the observed limb effect. He arrived at a redshift proportional to M/r . A similar dependence was predicted by Proisy²⁰ whereas Sadeh, Knowles and Au¹³ prefer M/r^2 . Mo and Pappas²¹ have treated the propagation of a classical electromagnetic wave in a Schwarzschild field which is treated as an equivalent medium. They find amplitude variations proportional to $\exp(M/r)$. However, none of these authors gives theory as widely applicable as the one presented here, nor do they consider the production of low energy photons as a result of the energy loss.

I argued previously²² that the angular momentum and linear momentum cannot both remain invariant in curved space-time. The method used a classical description of the momenta as volume integrals of the Poynting vector and treated curved space-time as an inhomogeneous refractive medium. Although the predicted energy loss for a photon passing near the Sun is similar to equation (7) the over-simplified treatment of curved space-time makes the earlier results inaccurate.

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Observations of solar oscillations with periods of 160 minutes

OSCILLATIONS of the Sun with a period near 160 min have been reported by Severny *et al.*¹ and further described by Kotov *et al.*². The presence of such oscillations would lead to a significant increase in our knowledge of solar physics. The reported amplitude is of the order of 1 ms^{-1} which is near the limit of observing technology. Observations described here at the Stanford Solar Observatory from 1975 to the present seem to support the reports from the Crimean Astrophysical Observatory^{1,2}.

At Stanford the relative velocity between a central circular area of radius $0.5R_{\odot}$ on the solar disk and most of the remaining area of the solar disk is measured. An optical scheme similar to that described in refs 1 and 2 is used and is described in detail by Dittmer³.

Light from the central portion of the solar disk is passed through a right circular polariser and light from the outer portion through a left circular polariser. The solar line Fe I $\lambda 5124 \text{ \AA}$ is observed and a Babcock magnetograph is used to measure the wavelength separation between left and right circularly polarised light, that is the difference between the velocity of the central part of the solar disk and the outer portion.

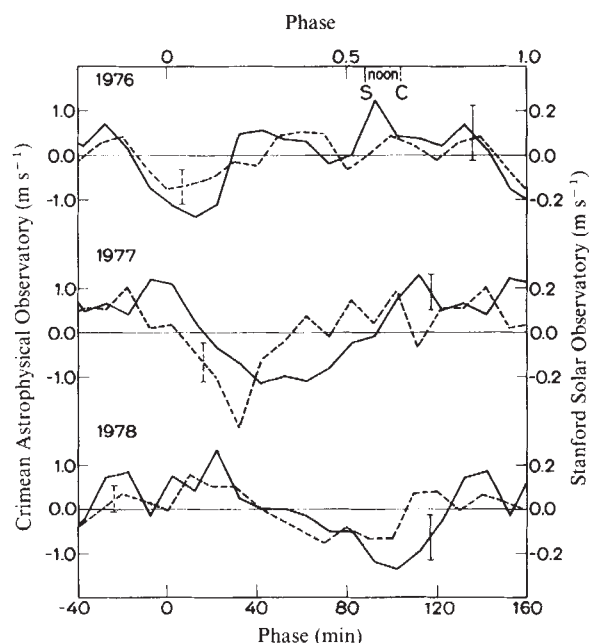


Fig. 1 Superposed epoch analyses with a period of 160 min for the observations during individual years at the Stanford Solar Observatory (solid line) and at the Crimean Astrophysical Observatory (dashed line) of the difference in velocity between the central portion of the solar disk and the outer portion.

Table 1 Summary of Stanford and Crimean observations

Year	Observatory	Dates	Days	Hours	Harmonic amplitude	Phase (min)
1974	Crimea	4 August–9 October	14	76	1.43	–22
1975	Crimea	28 June–14 October	28	157	0.89	–14
1976	Crimea	26 April–15 October	32	161	0.36	8
	Stanford	1 July–15 September	38	178	0.14	10
1977	Crimea	11 March–24 October	59	280	0.78	32
	Stanford	6 April–30 July	43	180	0.21	57
1978	Crimea	22 April–20 August	60	361	0.47	82
	Stanford	7 June–21 August	25	130	0.17	94

The fact that the period of 160 min is exactly one-ninth of a day requires that extreme caution be used in asserting that the observed oscillations are solar rather than terrestrial. Our observations considered as a continuous dataset lasting several months (or years) have considerable power at a period of 24 h because we do not observe at night and because there are drifts in the zero level through the day. At Stanford drifts in the guiding system are found to cause typically a 20 ms^{-1} drift in the signal baseline through a day. These drifts are removed by a parabolic fit. We describe here a superposed epoch analysis of the observations using a period of 160 min. This is equivalent to applying a filter that admits power corresponding to this period so that even if we have only noise input we will expect to receive a signal in the superposed epoch analysis. The comparison of observations between Stanford and the Crimea then rests largely on the relative phase of the observed oscillations at the two sites. If we both are observing a solar phenomenon we should expect to have the same phase within observational error, while if we are observing terrestrial phenomena or simply noise we should not expect phase agreement. During each of the years 1976, 1977 and 1978 the phase at the two observatories has been nearly the same, which supports the solar origin of the oscillations first reported at the Crimean Astrophysical Observatory^{1,2}. Table 1 gives the observing schedule during each year and the observed phase.

Figure 1 shows a superposed epoch analysis with period 160 min of the observations during each year at the Stanford Solar Observatory (solid line) and at the Crimean Astrophysical Observatory (dashed line). Negative velocity represents expansion of the Sun (motion of the central part of the disk towards the observer, note that this is the opposite of the convention used in previous Crimean publications on this subject) and the error bars are twice the standard error of the means. The zero phase in Fig. 1 is at 0 h UT.

Figure 1 shows that in each of the three years the phase at the two observatories is nearly the same. Figure 1 also shows changes in amplitude from year to year that are about the same for both observatories. On a time scale of months there also seems to be similar changes in amplitude at the two observatories, but due to the small amount of simultaneous observations (due to weather and observing schedules) the significance and origin of these variations is still uncertain.

We compute based on instrumental parameters (the size of the portion of the solar disk that is compared with the central portion of the disk) that the lowest mode of solar oscillation would be observed at the Crimea with twice the amplitude of the Stanford signal. The remaining factor of about two shown in Fig. 1 between the amplitude of oscillations observed at the Crimea and at Stanford is not understood.

Figure 1 shows an apparent drift in phase from one year to the next. A drift in phase computed with a fixed period is the same as an error in the period. This suggests that 160.0 min may not be exactly the correct period. Figure 2 shows the phase for all the yearly sets of observations available from both observatories. The fact that the points from both observatories tend to fall nearly on a straight line suggests that both sets of observations show solar motions. From the slope of a best fit line we compute a drift in phase of about 33 min per yr corresponding to a period of about 160.01 min.

An unknown terrestrial effect related to the time of local noon could yield an agreement in phase between the two observatories if they were separated in longitude by an integral number of $360^\circ/9$ intervals, as we are dealing with a period of one-ninth of a day. In fact the two observatories are separated by 3.9 such intervals, such that Stanford would have a phase 16 min earlier than the Crimea. In the three years in which we have observed, Stanford tends to have a phase later than the Crimea.

The apparent agreement in phase between the two observatories as well as the fact that the period seems to differ slightly from exactly one-ninth of a day tends to support the interpretation that we are observing solar oscillations. The dependence of the amplitude of the oscillations on the phase of solar rotation recently observed in the Crimea³ is also encouraging in this respect. Subject to the results of further years observations our present working hypothesis is that the oscillations are solar and that we can proceed towards further study of the modes involved and of the implications for our understanding of the Sun's interior.

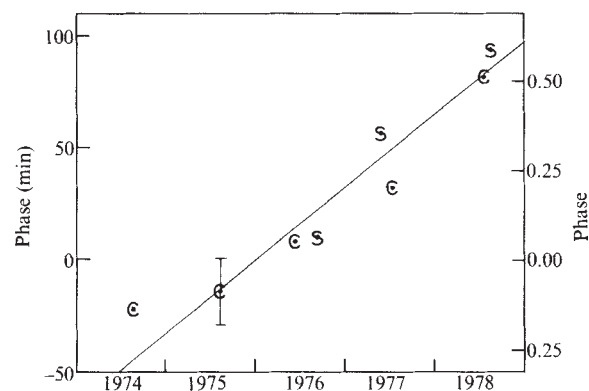


Fig. 2 Time of maximum expansion in minutes from 0 h UT for Stanford Observations (S) and Crimean Observations (C). The phase is computed from a harmonic analysis. The error bar shows the typical uncertainty in phase.

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Photoelectrocatalytic reduction of carbon dioxide in aqueous suspensions of semiconductor powders

WE report here the photoelectrocatalytic reduction of carbon dioxide to form organic compounds such as formic acid, formaldehyde, methyl alcohol and methane, in the presence of photosensitive semiconductor powders suspended in water as catalysts. Photocatalytic reaction kinetics were elucidated by reference to the theory of charge transfer at photoexcited semiconductors.

Heterogeneous photocatalytic reactions have recently been used to synthesise chemical compounds, thereby converting solar energy to chemical energy^{1–4}. Schrauzer and Guth have reported the photocatalytic reduction of nitrogen on a catalyst of wet TiO₂ powder. Oxygen and hydrogen were formed through the decomposition of water, and ammonia and/or hydrazine were also obtained through the photo-assisted synthesis of nitrogen and water on the TiO₂ catalyst³. Semiconductor photoelectrodes have been much investigated, with emphasis on electrochemical photocells converting solar energy to chemical and/or electrical energy^{5,6}. Photogenerated carriers (electrons and holes) in the space charge layer of a semiconductor electrode can transfer to the reactants in an electrolyte solution, and cause the photoassisted decomposition or synthesis of chemical compounds^{7,8}. Semiconductor catalysts may be assumed to undergo charge transfer across a solid-liquid junction similar to that in semiconductor electrodes; that is, charge transfer between semiconductor and redox reagents in solution would take the form of a conduction band and a valence band. Hemminger *et al.* have reported that methane was formed through the photosynthetic reaction of gas phase water and carbon dioxide molecules adsorbed onto strontium titanate surfaces in contact with platinum foil⁹. Recently Halmann has reported that carbon dioxide in an aqueous solution was reduced to formic acid at the p-type GaP photocathode in the electrochemical photocell with a fairly high conversion efficiency¹⁰.

In this study we attempted the photocatalytic reduction of carbon dioxide on various semiconductor photo-catalysts such as WO₃, TiO₂, ZnO, CdS, GaP, and SiC (99.5–99.9999% purity). In each case, semiconductor powder (200–400 mesh)

1.0–2.0 g was suspended in the 100 ml of purified water in a glass cell. Highly purified carbon dioxide gas was bubbled at 3 litres per min through a water-suspended catalyst shaken by a magnetic stirrer. The suspensions were illuminated, through a quartz window in the glass cell, by a 500 W Xe lamp or a 500 W high pressure mercury arc lamp. Illumination wavelengths were selected with cutoff filters. Products of the photocatalytic reactions were analysed by gas chromatography (Hitachi 163) using polyethyleneglycol (PEG) 200 and molecular sieve as column.

Table 1 Summary of yields of reduced products of carbon dioxide on various semiconductor catalysts

Catalysts (1.0 g/100 ml water)	Illumination period (h)	Yields of products	
		HCHO (× 10 ⁻³ M)	CH ₃ OH (× 10 ⁻⁴ M)
TiO ₂	7.0	1.1	2.3
TiO ₂	14.0	1.8	7.8
TiO ₂	30.0	1.8	14.6
TiO ₂ [*]	7.0	0.0	0.0
ZnO	7.0	1.2	3.5
CdS	7.0	2.0	11.7
GaP	7.0	1.0	11.0
SiC	7.0	1.0	53.5
WO ₃	7.0	0.0	0.0

Electrodes (–1.5 V vs SCE)	Amount of charges (C)	Yields of products	
		HCHO (× 10 ⁻³ M)	CH ₃ OH (× 10 ⁻⁴ M)
TiO ₂ [†]	108	1.7	11.7
TiO ₂ [‡]	180	2.8	56.7
p-GaP§	240	3.6	73.0

* TiO₂ suspension was illuminated with light of wavelengths longer than 500 nm.

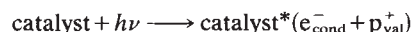
† TiO₂ electrode in 50 mM H₂SO₄ aqueous solution was not illuminated.

‡ TiO₂ electrode in 500 mM H₂SO₄ aqueous solution was not illuminated.

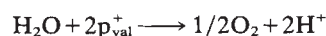
§ GaP electrode in 500 mM H₂SO₄ aqueous solution was illuminated with light of wavelengths shorter than 500 nm.

Formic acid, formaldehyde, and methyl alcohol were produced through the photocatalytic reduction of carbon dioxide in aqueous suspensions of semiconductors such as TiO₂, ZnO, CdS, GaP and SiC, when the suspensions were illuminated with light which was absorbable for the catalyst. A trace of methane was also detected in the gaseous products, which were trapped with liquid nitrogen refrigerant. Results of quantitative analysis of yields of reduced compounds from photocatalytic reduction of carbon dioxide in aqueous suspensions are given in Table 1. When the experiments were carried out in the dark or under illumination with a light not absorbed by semiconductor catalysts, no reduced products of carbon dioxide were detected with any of the semiconductor catalysts tested; as an example the results with TiO₂ are shown in Table 1.

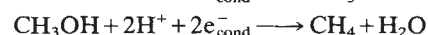
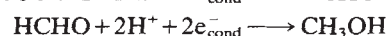
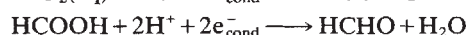
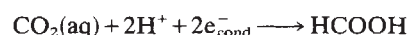
The photocatalytic reactions are considered to follow the schemes.



for an oxidation



for reductions



where e_{cond}^- and p_{val}^+ denote an electron in the conduction band and a positive hole in the valence band, respectively.

Reduced compounds such as formaldehyde and methyl alcohol were best produced on SiC semiconductor catalyst after

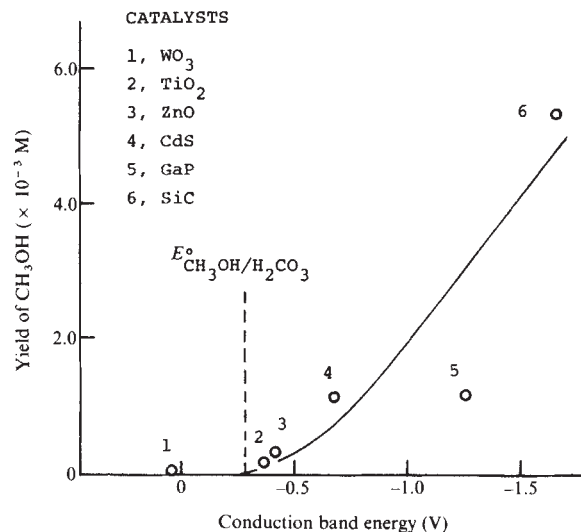


Fig. 1 Correlation between the yield of CH_3OH and the conduction bands of semiconductor catalysts. Dashed line denotes the standard redox potential of a $\text{CH}_3\text{OH}/\text{H}_2\text{CO}_3$ couple. Potentials are expressed against the normal hydrogen electrode (NHE).

illumination for 7 h, as shown in Table 1. Figure 1 shows the yields of methyl alcohol by photocatalytic reduction of carbon dioxide as a function of the energy levels of the conduction band of the semiconductor catalysts. The yields of methyl alcohol increase as the conduction band becomes more negative with respect to the redox potential of $\text{H}_2\text{CO}_3/\text{CH}_3\text{OH}$, whereas methyl alcohol was not produced in the presence of WO_3 catalyst which has a conduction band more positive than the redox potential of $\text{H}_2\text{CO}_3/\text{CH}_3\text{OH}$.

In experiments with semiconductor electrodes, carbon dioxide was reduced electrolytically to formic acid, formaldehyde, methyl alcohol and methane at the unilluminated TiO_2 electrode or at the illuminated p-type GaP electrode when both semiconductor electrodes were polarised at a potential of -1.5 V against a saturated calomel electrode for 2 h (Table 1). Thus electrons in the conduction band of semiconductor electrodes are able to reduce carbon dioxide in an aqueous solution.

It has been suggested that at semiconductor electrodes, the charge transfer rates between photogenerated carriers in semiconductors and the solution species depend on the correlation of energy levels between the semiconductor and the redox agents

in the solution¹¹⁻¹³. That is, the solution species of redox potential the more positive with respect to the conduction band level is better reduced at semiconductors. This hypothesis is illustrated in Fig. 2 which shows the energy correlation at the semiconductor-solution junction. When estimating charge transfer it is assumed that for semiconductor catalysts, as for semiconductor electrodes, photo-excited electrons in the more negative conduction band have the greater ability to reduce carbon dioxide in the solution.

Thus the photocatalytic reaction on a semiconductor catalyst appears to take place through the action of the photogenerated carriers (electrons and holes) acting as components of a local cell—reduction by electrons occurring in a conduction band and oxidation by holes in a valence band. The estimated quantum yields of production for photocatalytic reduction of carbon dioxide were $\sim 5.0 \times 10^{-4}$ for HCHO and 1.9×10^{-4} for CH_3OH with a TiO_2 catalyst, and 5.0×10^{-4} for HCHO and 4.5×10^{-3} for CH_3OH with SiC as catalyst, measured against the absorbable incident photons. We are now investigating the detailed dynamics of the photocatalytic reaction on the semiconductor catalysts.

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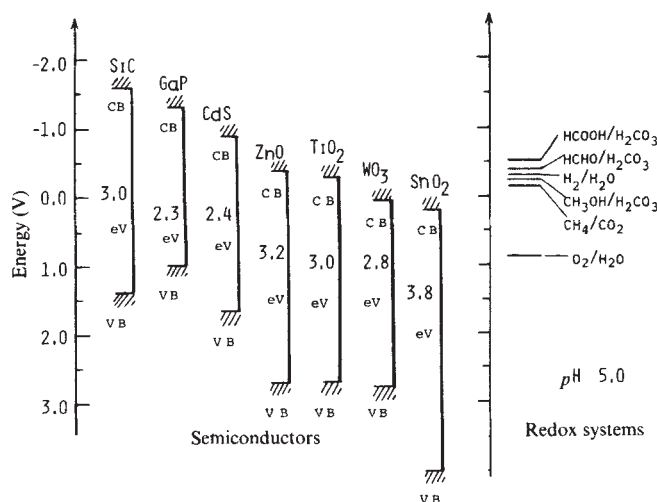


Fig. 2 A schematic illustration of the energy correlation between semiconductor catalysts and redox couples in water. CB and VB denote a conduction band and a valence band, respectively.

Highly efficient quantum conversion at chlorophyll *a*-lecithin mixed monolayer coated electrodes

THE design of solar conversion systems based on the photosynthetic primary reactions has attracted much attention recently. For instance, photoelectrolysis at a chlorophyll (Chl) *a*-water aggregate coated platinum electrode as a photocathode has been studied by Fong *et al.*¹⁻³, and simulation of the photoinduced electron transfer across the thylakoid membrane as well as of the biological ordered structure in a chloroplast has been attempted by Tien *et al.*^{4,5} using a Chl *a*-containing bilayer lipid membrane (BLM). We have already attempted to combine these different approaches⁶ by using a Chl *a* monolayer coated SnO_2 transparent electrode as a photoanode, and a maximum photocurrent quantum efficiency of 12–16% was attained with Chl *a*-stearic acid mixed monolayer systems. In that case, however, a decrease of the quantum efficiency was observed at Chl *a*-stearic acid molar ratios <1.0 ; this might be due to the formation of pheophytin *a* and/or to a possible heterogeneity of the mixed monolayers. We describe here how we have overcome such problems by using a phospholipid instead of the fatty acid as a

diluent for a Chl *a* monolayer. The hydrophobic interactions between Chl *a* and phospholipids have been well investigated both *in vivo* and *in vitro*, and the physical stability and photochemical activity of Chl *a*-synthetic lipid mixed layers were also confirmed in the BLM study⁷.

Chl *a* was prepared from spinach as previously reported⁶. Synthetic L- α -dipalmitoyl lecithin (DPL, Sigma) was used as a phospholipid without further purification. An SnO₂ optically transparent electrode (OTE)⁶ was used as a substrate of the monolayer deposition. The mixed monolayers of Chl *a* and DPL were prepared by spreading 2×10^{-4} M benzene solutions of Chl *a* and DPL in various molar ratios onto 10^{-3} M phosphate buffer solution (pH 7.5–8.0) in a Langmuir trough. A single layer of the mixed monolayer formed on the aqueous surface was deposited onto the SnO₂ OTE under the constant surface pressure of 20 dyn cm⁻¹. The electrochemical measurement setup was as reported previously⁶. The electrolyte solution used consisted of 0.1 M Na₂SO₄, 0.025 M phosphate buffer (pH 6.9), and 0.05 M hydroquinone as a reducing agent, and was flushed with nitrogen throughout the experiments. Monochromatic light from a 500-W Xe-arc lamp irradiated the monolayer coated SnO₂ OTE and the photocurrent was measured by a Keithley picoammeter (Model 417) in a potentiostatic condition of +0.1 V versus saturated calomel electrode (SCE).

The surface pressure-area (*F*-*A*) characteristic of Chl *a*-DPL mixed monolayer is compared in Fig. 1 with that of pure Chl *a*⁶. The stability of the mixed monolayer, even at pressures higher than 24 dyn cm⁻¹ (the collapse pressure of Chl *a* monolayer), implies that the present Chl *a*-DPL mixed monolayer could form a homogeneous two-dimensional solution. This homogeneity can be also supported by the observed change in the absorption property of the mixed monolayer following the dilution of Chl *a* content. On decreasing the molar ratio of Chl *a*-DPL from 1:0 to 1:99, the peak position of the red absorption band was found to shift gradually from 675 nm due to an aggregated state of Chl *a* towards 665 nm due to the monomeric state, accompanying the decrease in absorbance in proportion to the surface concentration of Chl *a*. These physical and spectral properties indicate the formation of homogeneously mixed monolayer as reported for other Chl *a*-diluent systems^{8,9}.

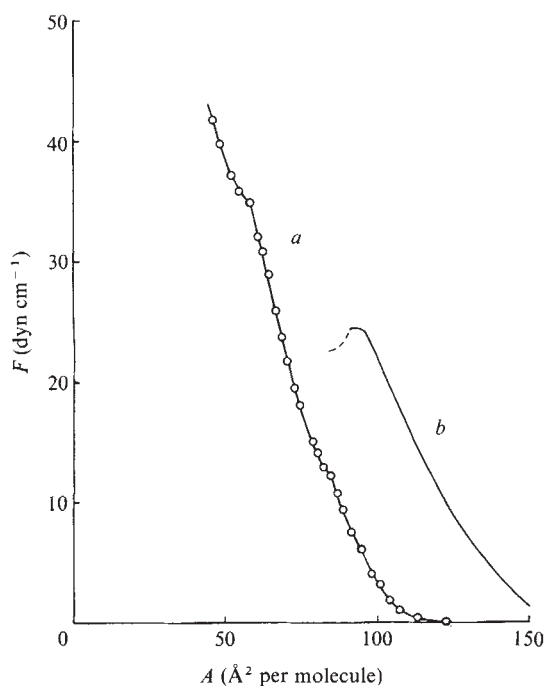


Fig. 1 *F*-*A* characteristics of a Chl *a*-DPL (1:1) mixed monolayer and a Chl *a* monolayer: *a*, Chl *a*-DPL (1:1) mixed monolayer; *b*, pure Chl *a* monolayer. Aqueous phase, 10^{-3} M phosphate buffer (pH 7.5); air atmosphere at 25°C.

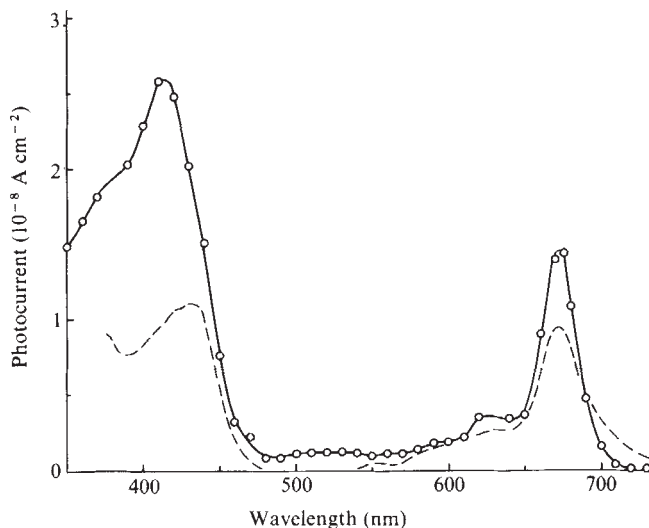


Fig. 2 Photocurrent spectrum at SnO₂ OTE deposited with mixed monolayer of Chl *a*-DPL (1:9). Dashed curve indicates the absorption spectrum of the mixed monolayer at the OTE-electrolyte interface. Composition of the electrolyte solution, 0.05 M hydroquinone, 0.1 M Na₂SO₄, and 0.025 M phosphate buffer (pH 6.9); electrode potential 0.1 V versus SCE; incident flux of monochromatic light, 2×10^{14} photons s⁻¹ cm⁻².

When a Chl *a*-DPL mixed monolayer was irradiated in the electrochemical cell, an anodic photocurrent was always obtained in accordance with the electron injection from excited Chl *a* into the conduction band of SnO₂. A typical photocurrent spectrum, corrected for the slight background photocurrent of SnO₂, for the mixed monolayer of molar ratio Chl *a*-DPL of 1:9 is shown in Fig. 2. The magnitude of photocurrent corresponds to a constant incident flux of 2×10^{14} photons s⁻¹ cm⁻² at each wavelength. The photocurrent spectra coincided well with the absorption spectra of mixed monolayers at OTE-electrolyte interface. The quantum efficiencies of photocurrents were determined in terms of electrons flowing per absorbed photon, the latter could be estimated from the incident photon flux measured by chemical actinometry and from the monolayer absorbance at an OTE-electrolyte interface. The photocurrent quantum efficiencies obtained with mixed monolayers at different molar ratios are listed in Table 1, together with the calculated mean intermolecular distances between Chl *a* molecules. As the Chl *a*-Chl *a* intermolecular distance increases, the photocurrent efficiency also tends to increase, with a hypso- and bathochromic shift of the photocurrent peak positions in the red and blue regions, respectively. Such an enhancement of the quantum efficiency is attributable to the suppression of intermolecular energy transfer between Chl *a* molecules by the increase in its intermolecular distance, because the energy transfer inevitably accompanies an energy loss which eventually decrease the overall efficiency of electron injection from excited Chl *a* to SnO₂. The concomitant shifts of photocurrent peak positions are compatible with the shifts in absorption peaks described above, and the absence of the characteristic peaks due to pheophytin *a* around 400–410 nm and 500–540 nm (ref. 10) in both photocurrent and absorption spectra of mixed monolayers indicates that the pheophytin formation is effectively suppressed in the present systems; a similar stability against the pheophytinisation was recently investigated by Iriyama (ref. 11 and personal communication).

The maximum efficiency of $25 \pm 5\%$ obtained in this work at neutral pH is relatively large, and almost twice that attained with the Chl *a*-stearic acid system⁶. Moreover, an examination of the pH dependence of the photocurrent showed that the quantum efficiency could reach $30 \pm 5\%$ when the experiment was done in the electrolyte solution at pH ~5. These values are sufficiently larger than the quantum conversion efficiencies reported (10^{-3} – 10^{-1}) (refs 3, 4, 12–14) with other solar conversion cells using Chl *a* as a photoreceptor.

Meilanov *et al.*¹⁵ in their attempt to verify the semiconductor model of photosynthesis, found a relatively high quantum efficiency (12–20%) for bulk photoconductive effects in metal-sandwiched dry Chl layers (0.3–2 μm thick) across which a high voltage (30–250 V) was applied. Our system is essentially different in that the photocurrent is produced by a direct electronic coupling between an excited Chl and the conduction band of SnO_2 , not by an accelerating effect of the electric field across the Chl layer.

An extension of the present experiments to the construction of biomimetic membranes on SnO_2 OTE by incorporating other photosynthetically important compounds into the Chl *a*-DPL mixed monolayer would be highly promising in view of the *in vitro* simulation of the photosystem II in plant photosynthesis.

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Enhanced CO_2 greenhouse to compensate for reduced solar luminosity on early Earth

CURRENT models for the evolution of the Sun require an increase in solar luminosity by 25% since the formation of the Solar System¹. Such an increase in the solar constant should have profound effects on the terrestrial climate, but there is no evidence from the fossil record of a corresponding change in the Earth's global mean temperature². This apparent conflict cannot be explained by the apparent inability of solar models to account for the low observed neutrino flux³. Even models that are forced to fit the neutrino data require a similar increase in the solar luminosity. As Newman and Rood¹ state: "a faint young Sun is one of the most unavoidable consequences of stellar structure considerations". We discuss here whether CO_2 - H_2O in a weakly reducing atmosphere could have caused this change in the early Earth's temperature by the so-called greenhouse effect.

Sagan and Mullen⁴ suggested that the solution must lie in a more efficient atmospheric greenhouse effect on Earth during the period when the solar luminosity was low. To increase the greenhouse effect, it is necessary to change the atmospheric composition, or density, or both. Sagan and Mullen⁴ postulated that the atmosphere was reducing during the early history of the Earth and found that a small amount of ammonia in the atmos-

phere (mixing ratio 10^{-5} – 10^{-6}) would provide the necessary opacity to outgoing infrared (IR) radiation to increase the mean surface temperature. In this model, the transition from reducing to oxidising conditions with the replacement of the NH_3 - H_2O greenhouse by a CO_2 - H_2O greenhouse comes about as a result of the development of widespread green plant photosynthesis 1,000–2,000 Myr ago. A similar route was invoked by Hart⁵, who developed a more detailed model for the evolution of the atmosphere.

We agree that an enhanced greenhouse effect caused by a change in atmospheric composition is a good solution to the solar constant dilemma, but we think the NH_3 - H_2O model is unrealistic. Current thinking about the early atmosphere of the Earth is shifting away from the traditional view that called for a highly reduced mixture of methane, ammonia and hydrogen, for reasons summarised by Walker⁶. More recent data have simply added strength to Walker's conclusions. As the noble gases on Mars show the same pattern of relative abundances as they do on Earth and in the 'planetary component' of the meteoritic gases, whatever fractionation process caused this pattern must have occurred before planet formation⁷. This being the case, the cosmic abundance ratio of 1.6×10^4 for H/Ne⁸ gives the maximum amount of hydrogen available for a 'captured' primitive atmosphere. Using the present atmospheric partial pressure of Ne (12.7×10^{-6} bar), the maximum hydrogen partial pressure on the primitive Earth from this source was 10 mbar. As Walker⁶ has pointed out, such an atmosphere would have dissipated in $<10^4$ yr. The partial pressure of ammonia in this atmosphere would have been comparable to that of neon, but its lifetime against photodissociation would have been short and it would have been out of equilibrium with crustal rocks.

Photochemical calculations by Kuhn and Atreya⁹ indicate that ammonia would be irreversibly converted to N_2 on the primitive Earth in <40 yr if the mixing ratio were $\leq 10^{-4}$. Eugster¹⁰ has investigated the stability of ammonia on the primitive Earth and concluded that a mixture of N_2 and H_2 will predominate over ammonia in a normal crustal environment. Outgassing of ammonia after the last phase of accretion (or after differentiation, choosing either an inhomogeneous or homogeneous model for planet formation) seems precluded by the lack of free iron in the upper mantle⁶. As life must have begun after this phase in the planet's history, it seems reasonable to agree with Eugster¹⁰ that any initial ammonia in the atmosphere "was oxidised to nitrogen long before the origin of life". Small amounts of methane and ammonia may have been transiently present in later times as a result of meteoritic and cometary impact, but lunar evidence indicates that bombardment rates must have been close to their present low values $\sim 3,600$ Myr ago¹¹.

In fact, the idea that the chemistry preceding the origin of life must have occurred in highly reducing conditions supported the traditional view of the composition of the early atmosphere¹². But there have been sufficient demonstrations that pathways towards the formation of essential pre-life compounds can occur only in weakly reducing conditions for this argument to have lost its original force. For example, Abelson¹³ was able to produce amino acids from the UV irradiation of a mixture of CO_2 , CO , N_2 , H_2O and just 1–10% H_2 . This seems a much more reasonable composition for the post-accretion (or post-differentiation) atmosphere of the primitive Earth^{6,10}. It is especially unlikely that the ammonia-containing mixture invoked by Sagan and Mullen⁴ (or ammonia plus methane suggested by Hart⁵) would last for 2,000–3,000 Myr after formation of the planet. But can this weakly reducing atmosphere provide the required greenhouse effect? The answer depends on carbon dioxide and water vapour, as the other gases are ineffective absorbers of IR radiation.

We have tested this possibility by assuming that the concentration of CO_2 in the early atmosphere was much higher than the present value. We have coupled the evolutionary model for the CO_2 abundance used by Hart⁵ with a radiative-convective CO_2 -climate model. (We used the model as in Augustsson and

Table 1 Predicted global surface temperature, T_s , throughout the evolution of the Earth's atmosphere

Time (10^9 yr BP)	S (W m^{-2})	P_{CO_2} (bar)	T_s (K)	
4.25	1,039	0.31	310	(284)
3.5	1,096	0.070	296	(284)
3.0	1,133	0.033	293	(284)
2.5	1,171	0.018	292	(286)
2.0	1,209	0.0086	290	(286)
1.5	1,247	0.0029	288	(286)
1.0	1,284	0.00065	286	(285)
0.5	1,322	0.00032	287	(287)
0	1,360	0.00032	290	(290)

The solar constant, S , is taken to be $S = 1,020 \text{ W m}^{-2}$ 4,500 Myr ago and is assumed to increase linearly with time. The CO_2 surface pressure, P_{CO_2} , is from Hart⁵. Temperatures listed in parentheses refer to deletion, within the model, of weak CO_2 bands at 9–10, 12 and $18 \mu\text{m}$.

Ramanathan¹⁴ except that we do not include the $7.6 \mu\text{m}$ pressure-induced band of CO_2 in the present analysis, because of the lack of band absorption data for the large amounts of CO_2 considered here.) Hart's model comprises a numerical simulation of the evolution of the Earth's atmosphere over its 4,500 Myr history, accounting for time-dependent outgassing, the Urey equilibrium, the onset of photosynthesis, and crude atmospheric chemistry to develop the time-history of the CO_2 concentration. Hart also presents NH_3 and CH_4 concentrations but we do not believe that these gases would persist within the early atmosphere. Moreover, even if CH_4 were present in the early atmosphere, this constitutes a very inefficient greenhouse gas. Quite obviously Hart's attempt contains many modelling uncertainties, but at least it produces a first estimate for the variability of atmospheric CO_2 over geological time.

Although Hart⁵ has considered the greenhouse effect from enhanced CO_2 , as well as other gases, he uses grey gas radiation modelling. But the realities of accounting for enhanced CO_2 require a more detailed radiation model^{14,15}. Moreover, Hart has used a cloud radiation model which produces strong negative climate feedback, contrary to the findings of recent cloud-climate modelling studies^{16,17}. This occurs as Hart relates global cloud amount to atmospheric water-vapour content, such that his cloud amount increases with increasing planetary surface temperature. But recent model predictions suggest that the reverse occurs (see ref. 16). Hart has additionally neglected the IR opacity of clouds. Thus, in his model, a warmer planet produces an enhanced planetary albedo due to increased cloudiness, but with no compensatory IR modification, and for this reason his model gives negative cloud feedback.

In the present study, we ignore climate-induced changes in cloud-cover fraction, following the suggestion that the cloud albedo and IR modifications are compensatory¹⁷. Moreover, we do not attempt to incorporate ice-albedo feedback, as the extent of the polar caps seems to depend on ocean advection and, in turn, the positions of the continents which have changed considerably over geological time¹⁸. Even without this effect, there are substantial uncertainties in modelling ice-albedo feedback¹⁹. Our model mostly predicts global temperatures greater than those at present; neglect of positive ice-albedo feedback will simply produce an underestimate of these temperatures. We further assume that the Earth's surface albedo is constant and equal to its present value, ignoring changes caused by mountain building and surface vegetation.

The results of our modelling are summarised in Table 1. We have simply assumed a total atmospheric pressure of 1 bar; the predicted global temperatures are not particularly sensitive to this assumption. The solar constant was taken to be $1,020 \text{ W m}^{-2}$ (75% of the present value), 4,500 Myr ago.

The predicted global temperatures of Table 1 suggest that enhanced CO_2 concentrations could have been the sole mechanism for preventing glaciation of the Earth when there was reduced solar luminosity. Past global glaciation would be

implied by climate models in the absence of an increased atmospheric greenhouse²⁰. Within the confines of our model calculations, the Earth was never more than 4 K colder than at present. This, of course, does not account for glacial-interglacial oscillations which, on our time scales, comprise short-term climatic variability.

A detailed analysis of our model results revealed that some weak bands of CO_2 , which have been neglected in previous studies of this problem^{4,5}, contribute significantly to the surface warming. These comprise two bands at $12 \mu\text{m}$, two bands at $18 \mu\text{m}$, and two bands in the 9–10 μm region. Predicted global surface temperatures, deleting these weak bands within the model calculation, are included in parentheses in Table 1. Clearly, these weak bands are important for large CO_2 concentrations. In this context, the neglect of the $7.6 \mu\text{m}$ band in the present model would produce an underestimate of the computed surface temperatures by about 5–10 K for CO_2 surface pressures of the order of 0.1–0.3 bar.

Our results are quite insensitive to events occurring from 4,500 to 4,250 Myr ago. For example, if there were an initial global glaciation during this time period, due possibly to limited outgassed CO_2 , the CO_2 greenhouse effect at 4,250 Myr ago would be sufficient to melt the surface ice. To show this, taking the surface albedo for an ice-covered Earth to be 0.7, we estimate the corresponding global temperature 4,250 Myr ago to be 285 K, far too high for global glaciation to persist.

We conclude that there is no need for ammonia to be present in the early atmosphere to maintain moderate temperatures when the Sun's luminosity was low. Carbon dioxide alone is sufficient, provided the partial pressure of this gas approaches the values suggested by the evolutionary model of Hart⁵. In view of the huge amount of CO_2 that has been produced over geological time and our lack of knowledge about rates at which release and deposition occurred in primitive times, this does not seem an unwarranted assumption. In fact, more detailed calculations may permit us to place a limit on the amount of CO_2 that could ever have been present in the atmosphere at one time, as the full complement (some 70 bar) would have caused excessive warming of the Earth's surface. We are also pursuing applications to the atmosphere of Mars, where larger CO_2 abundances in primitive times might have provided a sufficient greenhouse effect to permit the flow of liquid water apparently required by the evidence for fluvial erosion on that planet's surface.

The transition to an oxidising atmosphere on Earth between 2,000 and 2,500 Myr ago, brought about by green-plant photosynthesis, is then not accompanied by a transition from an $\text{NH}_3\text{--H}_2\text{O}$ to a $\text{CO}_2\text{--H}_2\text{O}$ greenhouse, but rather represents a simple increase in free molecular oxygen with the concomitant disappearance of CO. The composition of the intermediate atmosphere, spanning ~2,000 Myr from the early escape of hydrogen to the onset of oxygen-producing photosynthesis, was probably very similar to the present atmospheres of Mars and Venus, two planets on which any living organisms (if present) apparently have not been able to influence atmospheric composition as they have on Earth^{21,22}.

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2,390 Myr Rb–Sr whole-rock for the Scourie dykes of north-west Scotland

THE Scourie dyke swarm intrudes the Lewisian Complex in north-west Scotland. The structural state of these basic dykes is used to subdivide the Lewisian into the pre-dyke 'Scourian' and post-dyke 'Laxfordian' complexes¹. The ~2,700 Myr (refs 2–4) pyroxene-granulite facies Scourian gneisses of the Assynt area contain undeformed NW-trending dykes. Gneisses and dykes, which underwent the ~1,900 Myr (refs 5, 6) Laxfordian event, are now deformed and metamorphosed at amphibolite facies. Previous K–Ar measurements for the Scourie dykes in the Assynt area of the Lewisian gave a minimum age for dyke injection of 2,200 Myr (ref. 7). Younger K–Ar ages were thought to reflect later Laxfordian overprinting. I report here a Rb–Sr whole-rock isochron age of $2,390 \pm 20$ Myr (2σ) and an initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratio of 0.7022 ± 0.0001 (2σ) for the Scourie dykes.

The Rb–Sr whole-rock method is in principle more suited to the problem of determining the emplacement age of the Scourie dykes. However, the range in Rb/Sr and measured $^{87}\text{Sr}/^{86}\text{Sr}$ ratios of the individual dykes is very small, and so any geological scatter in the data points will result in a large error in the 'fit of the isochron' and, hence, in the calculated age. Table 1 gives Rb–Sr whole-rock isotopic analyses for suites of samples from three Scourie dykes in the area of least post-2,700 Myr deformation and metamorphism at Scourie. The dykes at north and south Scourie Bay are fresh two-pyroxene dolerites⁸, but the Kylesku Dyke contains amphibole replacement of pyroxene⁶.

The ages and initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratios of these three dykes, presented in Table 2, are all within 2 sigma error limits. Statistical tests (Student's *t*-test) show that the slopes of the isochrons are not significantly different at 95% confidence limits. There is

no geological or petrological evidence to suggest that these dykes are not cogenetic or coeval. The 33 data points combined define an isochron (MSWD = 2.1) which gives an age of $2,390 \pm 20$ Myr (2σ) and an initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratio of 0.7022 ± 0.0001 (2σ) (Fig. 1). This low initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratio is within the range thought characteristic of rocks derived from upper mantle source regions at ~2,400 Myr (ref. 9). The age from this isochron is interpreted as being close in time to the primary igneous crystallisation of the dolerite dykes in the Scourie region. Combining the three sets of data has increased the range in Rb/Sr ratio and considerably reduced the calculated error on the time of emplacement. This low ± 20 Myr uncertainty depends on the dykes being intruded at the same time and from the same source region. The combined Rb–Sr whole-rock age of $2,390 \pm 20$ Myr is considered the best estimate for the time of emplacement of the Scourie dykes investigated.

Table 1 Rb–Sr whole-rock isotopic analyses for samples from three Scourie dykes

Sample no.	Rb/Sr ($\pm 2\%$ ≤ 0.002)	Rb (p.p.m. $\pm 10\%$)	Sr (p.p.m. $\pm 10\%$)	$^{87}\text{Sr}/^{86}\text{Sr}$	$^{87}\text{Rb}/^{86}\text{Sr}$
'The Scourie Dyke', north Scourie Bay (GR 151455–144458)					
MC 64	0.116	16.2	139	0.71395 ± 8	0.336
MC 65	0.103	14.9	145	0.71265 ± 4	0.299
MC 66	0.168	25.0	148	0.71901 ± 6	0.488
MC 67	0.121	18.3	151	0.71432 ± 6	0.350
MC 68	0.115	17.2	151	0.71363 ± 6	0.331
MC 69	0.180	25.8	144	0.71997 ± 8	0.520
MC 70	0.170	25.5	149	0.71923 ± 4	0.492
MC 71	0.108	16.4	152	0.71295 ± 6	0.313
MC 72	0.148	22.7	153	0.71717 ± 6	0.429
MC 73	0.154	22.7	147	0.71731 ± 4	0.445
MC 74	0.161	24.3	151	0.71806 ± 4	0.466
MC 75	0.122	18.0	148	0.71440 ± 4	0.352
Kylesku Dyke (GR 234330)					
MC 76	0.0920	15.3	166	0.71149 ± 6	0.266
MC 77	0.1025	17.1	166	0.71221 ± 4	0.297
MC 78	0.0930	15.2	164	0.71131 ± 4	0.269
MC 79	0.0725	13.0	179	0.70926 ± 4	0.210
MC 80	0.1030	18.1	176	0.71246 ± 3	0.298
MC 81	0.0855	12.1	141	0.71074 ± 8	0.247
MC 82	0.0930	13.1	142	0.71163 ± 4	0.269
MC 83	0.0745	12.5	169	0.70980 ± 4	0.216
MC 84	0.0810	13.4	166	0.71038 ± 4	0.234
MC 86	0.0915	15.2	166	0.71149 ± 4	0.265
MC 87	0.0935	15.1	161	0.71168 ± 6	0.271
'Scourie Graveyard Dyke', south Scourie Bay (GR 148448)					
MC 13	0.0385	7.3	185	0.70600 ± 6	0.111
MC 14	0.0395	8.5	215	0.70594 ± 6	0.114
MC 15	0.0255	4.1	160	0.70458 ± 4	0.074
MC 16	0.0460	6.9	150	0.70683 ± 4	0.133
MC 17	0.0520	7.9	151	0.70708 ± 4	0.150
MC 18	0.0515	7.7	149	0.70757 ± 8	0.149
MC 19	0.0460	6.7	146	0.70696 ± 8	0.133
MC 20	0.0420	7.3	173	0.70641 ± 6	0.122
MC 21	0.0460	6.5	142	0.70665 ± 6	0.133
MC 22	0.0350	5.7	164	0.70569 ± 4	0.101

Rubidium and strontium were separated from the 3–5-kg samples by the standard techniques used in this laboratory^{6,17}. Isotope analyses were made on a 12-inch solid-source mass spectrometer using single Ta filaments¹⁸. Sr blank was always better than 6 ng and Rb blank less than 2 ng. Rb/Sr ratios were determined by X-ray fluorescence using extended counting times (not less than 400 s) on both peaks and backgrounds. The precision on duplicate analyses was always better than 2% or ± 0.002 (whichever is the greater) and were checked against isotope dilution analyses. The value of $1.42 \times 10^{-11} \text{ yr}^{-1}$ was used for the ^{87}Rb decay constant¹⁹. All errors are quoted at the 2 sigma level.

The main Scourian event formed granulite facies gneisses and NE–SW-trending structures. A later phase of NW–SE folding and associated almandine–amphibolite metamorphism has been recognised in the Assynt area^{10–12}. This younger Inverian¹⁰ event was synchronous with or occurred immediately before the intrusion of the Scourie dykes. The $2,390 \pm 20$ Myr date for the emplacement of the Scourie dykes provides a minimum age for this Inverian event. This is significantly older than the 2,200 Myr age previously quoted for this event^{7,10–16} and perhaps indicates that the Inverian was a late phase of deformation and retrogressive metamorphism in the major Scourian event.

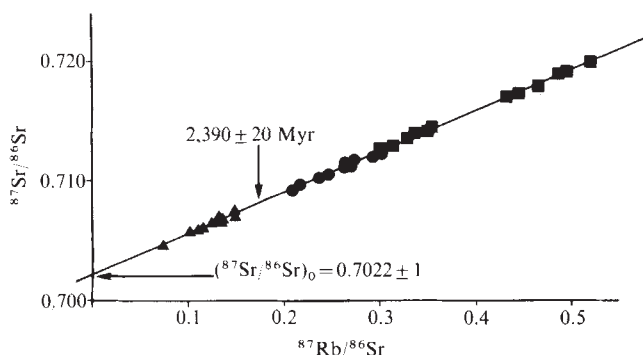


Fig. 1 Rb–Sr whole-rock combined isochron of samples from 'The Scourie Dyke' (■), Kylesku Dyke (●), and 'Scourie Graveyard Dyke' (▲). This isochron (MSWD = 2.1) gives an age of $2,390 \pm 20$ Myr (2σ) and an initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratio of 0.7022 ± 1 (2σ).

Table 2 Ages and initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratios of three Scourie dykes

Locality	No. of data points	Age (Myr)	$(^{87}\text{Sr}/^{86}\text{Sr})_0$	MSWD
North Scourie Bay	12	$2,330 \pm 60$	0.7025 ± 3	1.4
Kylesku	11	$2,380 \pm 150$	0.7023 ± 6	2.3
South Scourie Bay	10	$2,580 \pm 220$	0.7018 ± 4	2.4
Mean (Fig. 1)	33	$2,390 \pm 20$	0.7022 ± 1	2.1

The $\sim 2,400$ Myr age for the emplacement of the Scourie dyke swarm indicates that the Scourian tectonic episode had ceased by the beginning of the Proterozoic. This area of early continental crust then remained stable until the Laxfordian metamorphic and deformational event.

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Geomagnetic intensity in Athens between 2000 BC and AD 400

MEASUREMENTS of the intensity in Athens of the geomagnetic field between 2000 BC and AD 400 are reported here. The material from which the intensity was determined consisted primarily of sherds from the Agora excavations. Some sherds, and a whole skyphos, from the collection of the Ashmolean museum at Oxford were also sampled. The ancient intensity of the Earth's magnetic field was inferred from stepwise thermal demagnetisation. This is essentially the technique developed by Thellier¹, with the modifications described by Walton².

The magnetisation was measured with a Squid magnetometer which was essentially that described by Walton². Of the alterations to the apparatus the major change was to move the heating oven and field coils to a position below the magnetometer. The manipulations of the specimen were accomplished pneumatically. These were controlled by a small computer which also processed the signal from the Squid, printing out the components of the magnetisation, $\mathbf{M}$.

The laboratory field, $\mathbf{H}_L$, was applied in the vertical direction and, as described by Walton², an attempt was made to minimise the change in the vertical component of $\mathbf{M}$, $\mathbf{M}_z$. In practice, the choice of field was within 10–20% of the correct value. Accordingly, values of $\mathbf{M}_z$ were obtained both with a laboratory field applied, $\mathbf{M}_z^H$, and with no field applied $\mathbf{M}_z^0$. The values of $\mathbf{M}_z^H$ were then plotted against $\mathbf{M}_z^0$ and the laboratory field corrected

to give that field which would have produced a stationary value of $\mathbf{M}_z^H$. Values were also obtained for the transverse component, $\mathbf{M}_y^H$ and $\mathbf{M}_y^0$. The values of $\mathbf{M}_y^0$ were plotted against $\mathbf{M}_z^0$, and the slope of the resultant line yielded the angle between $\mathbf{M}$ and $\mathbf{H}$. By dividing the corrected laboratory field, $\mathbf{H}$, by the cos of this angle the estimated value of the ancient field was obtained.

Measurements were made, using the above procedure at temperatures which were nominally 200, 250, 300, 400, 500, and 600 °C (the higher temperatures were often omitted if the sample had already demagnetised sufficiently at lower temperatures).

In addition, values of $\mathbf{M}_y^H$ and $\mathbf{M}_z^H$ were obtained at intermediate temperatures. $\mathbf{M}_z^H$ was then plotted against $\mathbf{M}_y^H$. As no component of $\mathbf{H}$ was present in the transverse direction, $\mathbf{M}_y^H$ provided a measure of the degree to which the sample had demagnetised. Thus the plot of $\mathbf{M}_z^H$ against $\mathbf{M}_y^H$ could also be used to correct $\mathbf{H}$. Both plots were used, and the value of $\mathbf{H}$ which was used was an average of the two.

Finally, the angle between $\mathbf{M}_y^0$ and some arbitrary direction in the horizontal plane was monitored. The aim of this procedure was to identify the presence of possible additional components of $\mathbf{M}$ introduced by reheating the pottery in antiquity.

Attic pottery is, on the whole, quite strongly magnetised, a magnetisation density of 10^{-3} e.m.u. cm^{-3} being typical. Thus in principle a correction for demagnetising fields due to sample shape should be applied. However, this correction would not amount to more than the difference between the value for a disk with the field applied along a diameter, or at right angles to it. As the thickness of the sample was approximately equal to the diameter this correction was negligible.

A potentially more serious problem arises from demagnetising effects due to the shape of the vessel itself affecting the moment produced during firing in antiquity. In an attempt to estimate the magnitude of this effect, samples were taken from the base, sides and handles of an Attic skyphos which had been fired between 440 and 425 BC. However, before the results could be assessed a more serious source of error had to be considered.

Rogers (personal communication) has found that pottery is anisotropic: it is easier to magnetise in a direction parallel to the plane of the pot, than it is at right angles. It is possible to correct for this effect if the anisotropy ratio—the ratio of the moment produced in the hard direction to that in the easy direction—is known. The angle between the easy plane of magnetisation and $\mathbf{H}$ is known, and if the angle between this plane and the ancient field $\mathbf{H}^A$ is known, these two angles can be deduced from the changes in direction of $\mathbf{M}$ which occur on magnetisation in the laboratory field. The derivation of this correction factor will be detailed elsewhere. It turns out that the correction is simplified considerably if the sample is orientated so that $\mathbf{H}$ is either parallel or antiparallel to the laboratory field.

The results obtained from the Attic skyphos were corrected in this way. The anisotropy ratio was adjusted to yield the best agreement between samples taken from the base and those from the side, this amounted to 0.9. For these samples, the shape correction, if any, would be less than the scatter in the data. Hence, no shape correction was made. Unfortunately, the anisotropy correction of the results for the handles proved impossible because of instrumental difficulties, and these had to be discarded.

Having done this it was impossible to identify any shape effect, except possibly for the handles. Results for the latter, however, could not be corrected for anisotropy, so an unambiguous identification of a shape effect was impossible. In any case it seems that the shape correction is less than other sources of error for samples from the side and base, and all samples used came from these locations.

The anisotropy ratio obtained from these measurements on the skyphos was used to correct the values obtained from the rest of the material. This correction amounted to between 3 and 7% (obviously it could not be larger than 10%). The corrected values for the ancient field obtained from each sherd are plotted

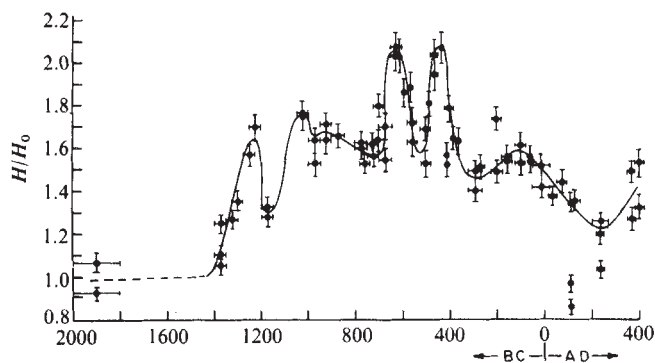


Fig. 1 The change in the ratio of the ancient intensity of the geomagnetic field H , to the present day value, H_0 in Athens. With the exception of the results on the skyphos each point represents a single measurement made on a core taken from a different sherd.

in Fig. 1. These results were obtained on about 80 sherds, and one whole vessel. Nine results were discarded because they displayed evidence of reheating or because alteration occurred before demagnetisation to a useful degree.

The horizontal error bars represent the archaeologists's estimate of the error in the date for the sherd in question. The vertical error bars were determined from the limits on the scatter of the results from the Ashmolean skyphos, namely $\pm 4\%$. This represents quite an arbitrary estimate of the error involved in obtaining the ancient field from a fragment of pottery. The error may, in fact, be larger than this. For one thing the degree of anisotropy is not necessarily the same. Nevertheless, with few exceptions the error bars for different sherds from the same time period overlap.

The exceptions fall into two categories. First, those sherds for which the field intensity was changing rapidly. In this case a difference in time of manufacture could lead to a difference in field $> 8\%$. Second, those sherds which did not, in fact, belong to the time period assigned, but were accidentally included. This is always a possibility with deposits.

Although anisotropy is potentially a large source of error, the 4% uncertainty in our results apparently mainly arises for another reason. This is clear from the scatter in the results obtained on the four cores from the base of the skyphos. The anisotropy correction for all of these must have been very nearly the same, but they still scatter by $\sim \pm 3\%$. In an earlier experiment² four slices from the same core yielded a scatter of $\pm 3.5\%$. The reason for the scatter seems to lie in nonlinearities in the plots of M_z^H against M_y^H or M_z^0 . These nonlinearities occur at both low and high temperatures. The high temperature deviations are undoubtedly caused by physical or chemical alteration of the magnetic minerals. The low temperature difficulties seem to be connected with the cooling rate. At low temperatures, below $\sim 250^\circ\text{C}$, it seems that the relaxation times involved are long enough for the moment produced to be significantly affected by the time at temperature, and the rate at which the sample is cooled.

Finally, another difficulty should be mentioned, there was clear evidence that some of the material had been reheated to a high temperature after the initial firing. This was particularly evident in the pottery produced after AD 200. Reheating may be responsible for the increased variability in the field values which is apparent in the results for this more recent material.

The question now arises as to whether the results display the actual change in the geomagnetic intensity or just some systematic variation in the fabric. If the former is true a similar change should be apparent in other locations. Thus, it is interesting to compare these data with those obtained by Bucha³ in Czechoslovakia. Although the details are not reproduced, the broad features of the two curves agree. In fact, Bucha's material would probably not be sufficient to show both peaks at 600 BC and 450 BC. Bucha⁸ also points out that because rotation of the core leads to a westward drift of roughly 0.2°yr^{-1} ; the maximum

should occur at about 0 BC in America and AD 500 in Japan. Bucha⁸ suggests that this is, in fact, apparent in measurements by Bucha *et al.*⁴ in Mexico and the southern US, and data obtained by Nagata *et al.*⁵ and Sasajima⁶ in Japan.

A change in the geomagnetic intensity with time such as that shown in Fig. 1 should be useful as an adjunct to dating. It is tempting to conclude on the basis of the data from Europe, America, and Japan that the broad features of the time dependence of the field intensity at other sites may be predictable, using these data and the rate of westward drift. However, such a conclusion would be premature as this field dependence on time does not occur in all results obtained by others. Nor, if it is present, does it always occur in a time period consistent with a rate of westward drift of 0.2°yr^{-1} (see ref. 7).

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Magnetic anisotropy in ancient pottery

WHILST measuring the thermoremanent magnetism (TRM) of ancient pottery to determine the field magnitude at the time of firing, by using a modification of the Thellier thermal remagnetisation technique¹, we have observed that the ease with which TRM is acquired is dependent on the angle at which the remagnetising field is applied. As a general rule it seems that the hardest direction is perpendicular to the plane of the pottery and that to a rough approximation there is an 'easy plane' lying in the plane of the pottery. The effect is apparently distinct from that due to the demagnetising field associated with sample shape, because the anisotropy effect is observed for sample shapes for which there would be no variation of demagnetising field with change of angle of applied field (such as a cylindrical sample being magnetised at various azimuthal angles in the plane perpendicular to the axis of the cylinder), as well as for samples for which the specific magnetisation is too weak for demagnetising fields to be significant. The anisotropy effect has been observed also in alternating field (750 Hz) measurement of instantaneous susceptibility.

Consideration of demagnetising fields suggests that the TRM acquired by a disk-shaped sample will be greater when the disk is orientated so that its plane is parallel to the field than when it is orientated perpendicular to the field. As reported previously², such a difference was observed for disks (diameter 25 mm, thickness 7 mm) of baked clay from a suite of samples for which the specific magnetisation ranged from 4×10^{-3} to $0.06 \times$

Table 1 Anisotropy data for various samples of ancient pottery

	Specific magnetisation ($\text{A m}^2 \text{ kg}^{-1} \times 10^3$)	Ratio of maximum to minimum TRM susceptibility
Romano-British coarse pottery (sample 559 a 1.2)	1.1	1.2
Gallo-Roman pottery (Samian ware)		
(561 a 2.1)	0.4	1.6
(1604 a 1.2)	0.3	1.7
(1604 a 4E)	0.3	1.7
Roman tile		
(1631 a)	2.2	1.2
(1631 92)	2.1	1.4
Minoan pottery (LMIA)		
(1635 a 1)	2.5	1.6
Recently fired 'ancient' pottery		
(1607 d.1.1)	3.3	1.7
(1614 c. 3.2)	3.6	1.1
Recent brick (IB4)	1.5	1.04

Note that the specific magnetisations quoted are for an applied field of $100 \mu\text{T}$ in the direction of minimum TRM susceptibility.

$10^{-3} \text{ A m}^2 \text{ kg}^{-1}$ (the numerical values are the same when specific magnetisation is expressed in $\text{G cm}^3 \text{ g}^{-1}$). Consideration of shape demagnetisation effects alone suggested that for the weakly magnetised samples the difference would be negligible; in fact, some of the weakly magnetised samples showed a difference which was comparable with, and in one case greater than, the 30% difference shown by samples at the upper end of the specific magnetisation range. The explanation for this unexpected result may lie in the intrinsic magnetic anisotropy of the baked clay fabric.

The intrinsic anisotropy was first directly observed in squat cylindrical samples ($3 \text{ mm} \times 3 \text{ mm}$) cored from Gallo-Roman pottery (Samian ware), using the SQUID cryogenic magnetometer of Walton³. In the modified⁴ version, the oven is mounted directly below the measuring cavity and the sample, mounted with fireclay on a vertical quartz rod, can be positioned either in the oven or in the cavity. The vertical component of its magnetisation is determined from the SQUID signal resulting from the flux change in the vertical pick-up coils during movement into (or out of) the cavity. The horizontal component is determined from the amplitude of the sinusoidal variation of flux picked up by the horizontal detection coils due to rotation of the sample about a vertical axis at around 2 revolutions per second; the vertical and horizontal detection coils are connected

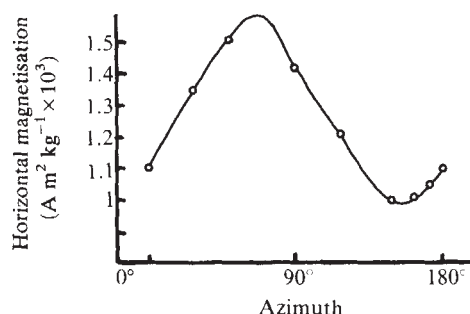


Fig. 1 Variation of TRM with direction of application of the applied field for a sample of Minoan pottery (LM 1A, sample no. 1635 a 1). The vertical scale shows the horizontal component of the TRM resulting from application of a horizontal field of $50 \mu\text{T}$; the field was applied while the sample cooled from 500 to 150°C . The sample was a $3 \text{ mm} \times 3 \text{ mm}$ cylinder cored with its axis perpendicular to the plane of the pottery. It was mounted for measurement with its axis horizontal and at zero azimuth on the scale shown; the orientation was only approximate and it is considered that the maximum is displaced from 90° because of this.

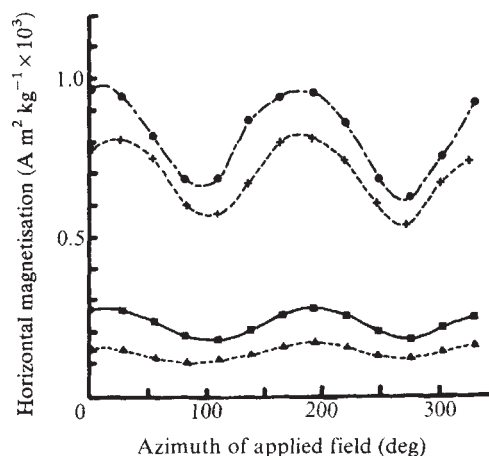


Fig. 2 Variation of TRM with direction of applied field for a sample cooled in a horizontal field of $50 \mu\text{T}$ over various temperature ranges. ●, $450\text{--}300^\circ\text{C}$; +, $300\text{--}150^\circ\text{C}$; ■, $150\text{--}20^\circ\text{C}$; ▲, $600\text{--}450^\circ\text{C}$. The data points through which the full line is drawn were obtained by cooling the samples from 150° to 20°C in the presence of the applied field. Similar curves were produced for different temperature ranges by cooling in the field from the higher temperature stated switching off the field at the lower temperature. The sample was a $3 \times 3 \text{ mm}$ cylinder mounted with its axis approximately vertical. It had been obtained by coring parallel to the plane of a piece of pottery (1607 d 1) that had been fired in AD 1962 in a replica of a Romano-British kiln at Boston, Lincs.

in series to the SQUID element itself. In the oven position, controlled vertical and horizontal steady fields can be applied to the sample; in their absence the residual Earth's field is $<0.5 \mu\text{T}$ because of mu-metal screening. Applied fields in the range $10\text{--}100 \mu\text{T}$ were used in the present work.

In Samian ware samples, for a sample cored with its cylindrical axis perpendicular to the plane of the pottery the TRM resulting from a field along the axis was 30–40% lower than the TRM resulting from the same field applied perpendicular to the axis. Conversely for a sample cored with its axis parallel to the plane of the pottery the TRM resulting from a field along the axis was 30–40% higher than for the field applied perpendicular to the axis. Table 1 lists results for this and other samples and Fig. 1 shows the variation of resultant TRM with angle of applied field for one of them. It has also been observed, for two samples at any rate, that the degree of anisotropy is the same for partial TRM's given over different temperature ranges; this is shown in Fig. 2.

A consequence of fabric anisotropy is that an applied field gives rise to a small TRM component transverse to the direction of the field unless the field is either exactly parallel or perpendicular to the 'easy plane'; if the applied field makes an angle α with the easy plane then the ratio of the transverse TRM component to the parallel component is given by

$$\frac{(a-1) \tan \alpha}{a + \tan^2 \alpha}$$

where a is the ratio of the TRM susceptibility within the easy plane to the susceptibility perpendicular to that plane, the susceptibility within the easy plane being taken as uniform. This transverse component has been observed and in Fig. 3 we show the vertical TRM component resulting from application of a horizontal field at various azimuths, zero azimuth being taken as the direction of the horizontal component resulting from an applied vertical field. The maximum of the vertical component occurs at this zero azimuth as expected from consideration of the easy plane model. In contrast, the azimuthal variation of the horizontal component (resulting from the applied horizontal field) is not as predicted from the model; this is because it is masked by variation of susceptibility within the easy plane. As mounted for the measurements of Fig. 3 the ratio of the average horizontal TRM susceptibility to the vertical susceptibility was

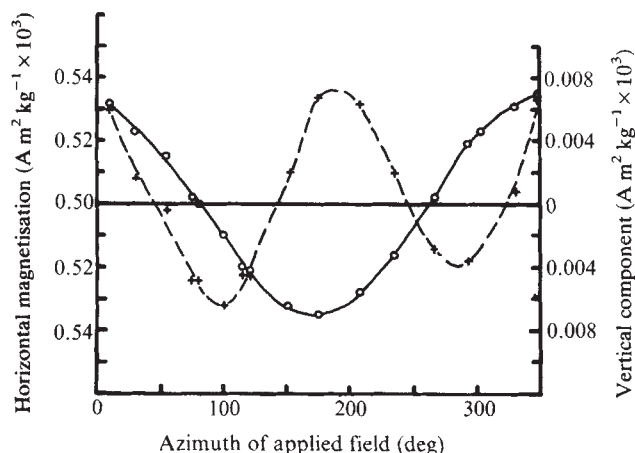


Fig. 3 Horizontal (+) and vertical (O) components of the TRM resulting from horizontal applied field of $100 \mu\text{T}$ applied at the azimuth shown, zero azimuth being taken as the direction of the horizontal component of the TRM that results from a vertical field; the field was applied while the sample cooled from 500° to 150°C . The sample was a $3 \times 3 \text{ mm}$ cylinder obtained from a piece of Samian ware (561 a.2.1) by coring perpendicularly to the plane of the pottery; it was mounted for measurement with its axis roughly vertical.

1.43 and application of a vertical field produced a horizontal TRM component which was 21% of the vertical component; these data indicate that the easy plane was inclined at an angle of $\sim 25^\circ$ to the horizontal and that the ratio of the average TRM susceptibility within the easy plane to the susceptibility perpendicular to it is 1.6.

The explanation that we propose for this intrinsic anisotropy in the fabric of pottery is that it is due to preferential alignment of magnetic grains. The alignment presumably occurs during the moulding of the clay into shape and that perhaps it is further enhanced by grain growth during firing. Whatever its cause the effect dictates that for reliable determination of ancient field by thermal remagnetisation it is important to apply the laboratory field in such a direction as to produce a TRM in the same direction as the magnetisation that was acquired in antiquity, or to make appropriate correction. Failure to do either could lead to an error of up to 60% in the case of the sample on which Fig. 1 is based, and to ensure that the error from misalignment is kept below 5% it is necessary to have the direction of the laboratory TRM within 10° of the direction of the ancient magnetisation, as will be seen from Fig. 1. Note that in the classic work of Thellier and Thellier¹ the samples concerned were from bricks, tiles and kilns; at any rate for the two former the data of Table 1 suggest that the effect may be relatively unimportant. The pottery listed in Table 1 is presumed to have been made on a wheel; however, the alternating field measurements included some non-wheel-made pottery of the British Iron Age and this too showed some effect. This and other aspects are being investigated further.

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Correlation between heart attacks and magnetic activity

SINCE the pioneering work of Düll and Düll¹, many papers have been published on the possible correlation between geomagnetic activity and medical phenomena. Most of them conclude that there is such a correlation, although this may reflect the tendency to publish positive results and discard negative ones, and there is widespread scepticism concerning the reality of the correlation. The main objections concern the validity of the statistical tests, failure to remove trend and inadequate allowance for seasonal variations. Here we present data for which the correlation is particularly high, and can be demonstrated convincingly by standard statistical tests.

The medical data consist of the number of cardiac emergencies admitted each day to the cardiac/thoracic wards of the main hospitals in Hyderabad and Secunderabad, the Osmania General Hospital and the Gandhi Hospital, respectively, during 1967–1972. Almost all the acute heart cases in those cities are admitted to the two hospitals except a few that are treated privately. The geomagnetic data consist of daily sums of Kp, the planetary index of geomagnetic activity for 3-hourly intervals of UT. The Kp index is intended to measure the geomagnetic effect of solar particle flux. Values of Kp are published, together with details of their definition and derivation, by the International Union of Geodesy and Geophysics². The daily sums of Kp cover an interval of 24 h starting from 18 h UT, whereas Indian Time starts at 18.5 h UT, so there is a small lag of 30 min between the geomagnetic and medical data. The interval 1967 to 1972 was selected before the data were examined.

The geomagnetic data show marked variations with season, and similar variations are seen in the medical data (Fig. 1). This is to be expected if they are correlated, but the seasonal variations in the medical data might equally be due to other (for example, meteorological or dietary) causes. To eliminate such seasonal effects, and also to avoid long-term trends (which are in any case small, Table 1), we have considered the data a month at a time.

Table 1 Annual mean values

Year	Kp sum	Daily admissions
1967	15.9	2.35
1968	18.4	2.72
1969	15.9	2.51
1970	16.1	2.10
1971	16.2	2.25
1972	16.8	2.49

Table 2 lists, for each month, the coefficient of correlation, r , between the medical, M , and geomagnetic, G , parameters, and also the constants a and b for the relation $G = aM + b$, obtained by the method of least squares. The results for the first few months are presented graphically in Fig. 2; as the correlation coefficients confirm, the remaining months are essentially similar. In each case, there is a clear tendency for the number of admissions to increase with magnetic activity.

To estimate the probability of such correlations occurring by chance, Student's t test would seem appropriate, but for this test to be valid, the data need to be approximately representative of a normal distribution, and both the G and M data are heavily biased towards the lower end of their range. The standard way of making such distributions more nearly normal is to take logarithms, and this is quite satisfactory for G , but for M (which includes zero values) a constant must first be added. We have chosen the constant to be 10, as this value approximately equalises the positive and negative moments about the mean.

Table 2 Data for each month

Month	<i>r</i>	<i>a</i>	<i>b</i>	<i>r'</i>	<i>P</i>	Month	<i>r</i>	<i>a</i>	<i>b</i>	<i>r'</i>	<i>P</i>	Month	<i>r</i>	<i>a</i>	<i>b</i>	<i>r'</i>	<i>P</i>
1967						1969						1971					
Jan.	0.77	3.6	5.5	0.71	2.1×10^{-3}	Jan.	0.56	2.6	7.8	0.60	1.4×10^{-2}	Jan.	0.79	3.8	6.7	0.61	1.2×10^{-2}
Feb.	0.80	3.8	5.1	0.79	7.7×10^{-4}	Feb.	0.39	2.1	11.7	0.32	2.6×10^{-1}	Feb.	0.66	3.1	10.3	0.55	4.2×10^{-2}
Mar.	0.68	3.1	5.7	0.60	1.4×10^{-2}	Mar.*	0.73	3.7	7.2	0.63	1.2×10^{-2}	Mar.	0.74	3.8	6.9	0.72	1.7×10^{-3}
Apr.	0.57	2.6	9.2	0.56	3.0×10^{-2}	Apr.*	0.36	1.4	16.5	0.30	3.0×10^{-1}	Apr.	0.64	2.6	12.1	0.62	1.4×10^{-2}
May	0.76	4.0	7.5	0.70	2.5×10^{-3}	May	0.76	4.6	6.8	0.67	4.5×10^{-3}	May	0.90	4.3	8.4	0.81	1.4×10^{-4}
June	0.61	2.0	10.9	0.62	1.4×10^{-2}	June	0.51	3.0	10.7	0.58	2.3×10^{-2}	June	0.76	4.6	8.8	0.69	4.4×10^{-3}
July	0.73	2.9	8.4	0.68	3.8×10^{-3}	July	0.70	3.6	5.8	0.71	2.1×10^{-3}	July	0.41	1.5	10.8	0.43	9.6×10^{-2}
Aug.	0.56	2.5	11.5	0.50	4.9×10^{-2}	Aug.	0.40	1.7	9.9	0.38	1.5×10^{-1}	Aug.	0.49	2.8	9.7	0.45	8.0×10^{-2}
Sep.	0.83	4.7	7.8	0.78	6.0×10^{-4}	Sep.	0.81	3.9	5.5	0.73	2.0×10^{-3}	Sep.	0.71	3.2	9.2	0.73	2.0×10^{-3}
Oct.	0.74	3.8	5.6	0.73	1.3×10^{-3}	Oct.	0.72	4.2	4.6	0.72	1.7×10^{-3}	Oct.	0.78	4.4	7.6	0.81	1.4×10^{-4}
Nov.	0.56	4.1	7.8	0.48	7.0×10^{-2}	Nov.	0.78	4.0	4.4	0.69	4.4×10^{-3}	Nov.	0.83	4.3	3.9	0.68	5.3×10^{-3}
Dec.	0.73	3.8	7.7	0.71	2.1×10^{-3}	Dec.	0.53	2.9	5.6	0.52	3.9×10^{-2}	Dec.	0.80	4.4	3.7	0.69	3.1×10^{-3}
1968						1970						1972					
Jan.	0.63	2.2	12.4	0.58	1.9×10^{-2}	Jan.	0.41	1.3	10.3	0.31	2.4×10^{-1}	Jan.	0.65	2.9	10.6	0.51	4.4×10^{-2}
Feb.	0.78	4.5	6.0	0.78	6.0×10^{-4}	Feb.	0.50	2.6	6.8	0.56	3.7×10^{-2}	Feb.	0.55	1.9	10.9	0.48	7.0×10^{-2}
Mar.	0.56	2.1	13.7	0.51	4.4×10^{-2}	Mar.	0.87	4.1	6.4	0.81	1.4×10^{-4}	Mar.	0.70	3.4	8.4	0.63	8.9×10^{-3}
Apr.	0.51	3.1	11.4	0.37	1.7×10^{-1}	Apr.	0.62	4.3	9.7	0.65	8.7×10^{-3}	Apr.	0.71	3.1	9.3	0.61	1.6×10^{-2}
May	0.74	3.2	11.5	0.68	3.8×10^{-3}	May	0.55	2.4	11.0	0.51	4.4×10^{-2}	May	0.72	2.7	10.2	0.70	2.5×10^{-3}
June	0.60	4.3	10.0	0.53	4.2×10^{-2}	June	0.85	5.1	7.5	0.79	4.6×10^{-4}	June	0.79	4.0	7.7	0.71	3.0×10^{-3}
July	0.63	2.8	11.6	0.57	2.1×10^{-2}	July	0.85	3.7	11.3	0.77	4.8×10^{-4}	July	0.32	1.6	9.6	0.23	3.9×10^{-1}
Aug.	0.60	2.7	9.7	0.55	2.7×10^{-2}	Aug.	0.82	4.4	7.5	0.77	4.8×10^{-4}	Aug.	0.85	4.7	7.5	0.77	4.8×10^{-4}
Sep.	0.81	3.9	7.8	0.80	3.4×10^{-4}	Sep.	0.69	3.6	10.0	0.58	2.3×10^{-2}	Sep.	0.63	3.3	8.4	0.65	8.7×10^{-3}
Oct.	0.77	4.2	4.8	0.72	1.7×10^{-3}	Oct.	0.65	3.3	9.4	0.55	2.7×10^{-2}	Oct.	0.64	3.8	7.7	0.68	3.8×10^{-3}
Nov.	0.77	5.2	1.1	0.67	6.3×10^{-3}	Nov.	0.73	3.0	9.8	0.62	1.4×10^{-2}	Nov.	0.77	4.0	7.0	0.71	3.0×10^{-3}
Dec.	0.68	2.3	8.9	0.69	3.1×10^{-3}	Dec.	0.77	3.1	6.8	0.69	3.1×10^{-3}	Dec.	0.61	3.2	6.4	0.58	1.9×10^{-2}

* Medical data are not available for 9 and 10 March, 11 and 12 April 1969.

The coefficient of correlation, r' , between $\log G$ and $\log (M + 10)$ is given in the penultimate column of Table 2. In general, r' is similar to but slightly less than r . The final column of Table 2 lists the probability of such a correlation occurring by chance, according to Student's t test, using $t = r'/[(1 - r'^2)/\nu]^{1/2}$, where ν denotes the number of degrees of freedom in the data for 1 month³. If the data for successive days were completely independent, ν would be $N - 2$, where N is the number of days in the month. However, it is certainly true in the case of magnetic activity, and may also be true for heart attacks, that the data show considerable conservation; that is, a high value is likely to be followed by another high value; and this may considerably reduce the effective number of degrees of freedom⁴. Following Taubenheim⁵, we find that, for our data, the effective number of degrees of freedom is $\nu = N/2.3 - 2$ for $\log G$ and $\nu = N/1.9 - 2$ for $\log (M + 10)$, and the second value of ν is that appropriate for significance tests. The values of P in Table 2 were deduced using the integral part of ν from the second relation.

From uncorrelated data we might expect 3 or 4 of the 72 correlation coefficients to achieve the 5% level of significance ($P < 5 \times 10^{-2}$), but we find that all except 10 are significant at this level. Similarly, we find 38 to be significant at the 1% level, and 11 at the 0.1% level, compared with 1 and 0 to be expected by chance.

Because there is no *a priori* reason to expect any conservation in the medical data, the presence of such conservation at a level similar to that in the geomagnetic data provides some additional support for the hypothesis that there is an association between the two phenomena. Also it should be noted that, although the adjustments for lack of normality and for conservation seem to complicate the analysis, had either of these been omitted, the level of significance would have appeared to be even higher.

Why should these data show such a high correlation, when those of Lipa *et al.*⁶ for the times of deaths in the United States due to coronary heart disease and stroke during 1962–1966 showed no significant correlation with magnetic activity? First, the level of artificial magnetic disturbance (from cars, industry, domestic appliances and so on) is very much lower in India than in the United States. Second, they considered the time of death, which may occur much later than the medical circumstance which was its cause.

It may be objected that our data and method of analysis are not the most appropriate. Some of the admissions of cardiac emergencies may have occurred a day or more after the onset of the condition, others may have been delayed for administrative reasons (for this reason, days of zero admissions were omitted from a previous study⁷). A proportion of the cardiac emergencies may have been diagnosed wrongly. Perhaps we should have used a local measure of magnetic activity, as was done by Srivastava *et al.*⁷, but, because one of us is responsible for the measurement of these, it was thought best to use an international parameter to avoid any suspicion of bias. (In any case, the difference between Kp and the local measure of K is always small.) Maybe a better result would have been obtained if we had introduced an appropriate time-lag between the magnetic and medical parameters. However, none of these possibilities would be expected to decrease the correlations, and we have analysed our data in the most straightforward way possible in the hope of convincing the reader that, in this case at least, there is a significant correlation between medical and geomagnetic phenomena.

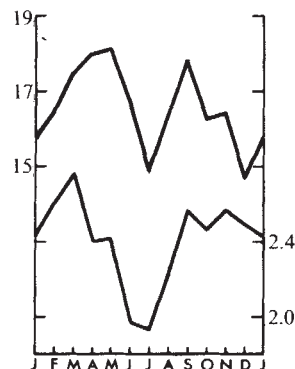


Fig. 1 Monthly mean values of the daily sums of magnetic activity indices, Kp (upper graph) and the daily admissions of cardiac emergency cases (lower graph).

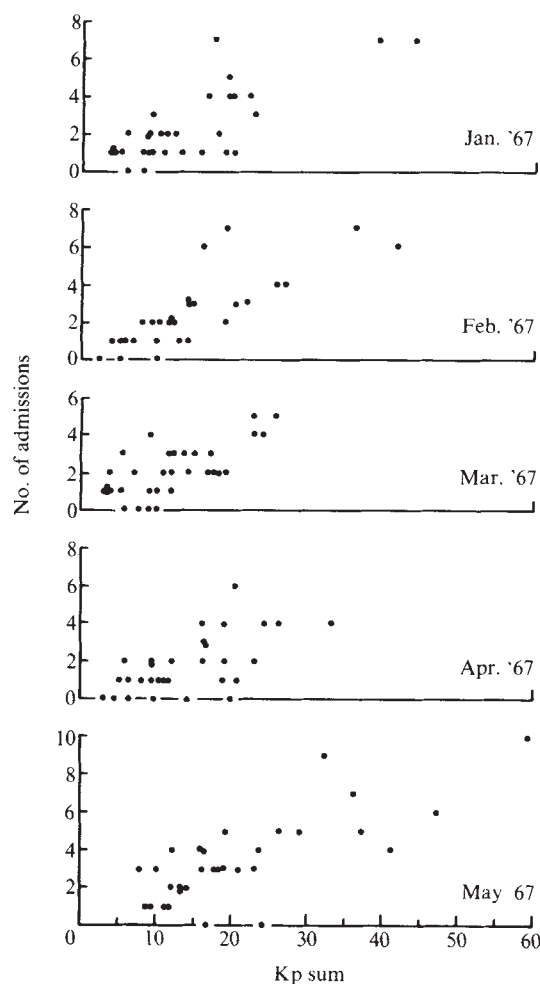


Fig. 2 Plots of daily admissions of cardiac emergency cases against daily sums of magnetic activity indices, Kp, for the first 5 months of data.

The possibility that there is some other cause (of solar origin?) responsible for both the magnetic and medical phenomena should not be ignored.

We thank the Superintendents of the Osmania General Hospital, Hyderabad, and the Gandhi Hospital, Secunderbad, for making available their hospital records, and our colleagues at the Hyderabad Magnetic Observatory for abstracting the medical data from these records.

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Evidence that geomagnetic variations can be detected by lorenzinian ampullae

THERE have been several reports of electroreceptor responses in elasmobranchs to an electric field induced by various magnetic stimuli (including water flow in a geomagnetic field)¹⁻⁶. The possibility was suggested that fish can detect telluric currents produced by geomagnetic variations⁷, but it was not investigated experimentally. Here we describe direct neurophysiological evidence for such a phenomenon.

Experiments were carried out on Barents Sea skates *Raja radiata* in Dalnje-Zelenetsk Bay, on the Barents Sea. Single-unit activity was recorded as previously described⁵ from nerve fibres supplying the ampulle of Lorenzini of the hyoid ampullary group. Impulses were processed by an integrator so as to follow the spike frequency in the course of the experiment.

The electrical state of the sea was monitored using two Ag-AgCl electrodes 400 m apart, parallel with the coastline. The two electrodes were connected by a marine cable to similar electrodes in the experimental tank. The electrodes in the tank were 1 m apart. To equalise the voltage gradients in the sea and the tank, two resistors (R_1 and R_2) were interposed, as shown in Fig. 1. The resistors were chosen by the following reasoning. Voltage gradient in the tank is given by

$$E_t = E_s \frac{l_s R_t}{l_t (R_1 + R_2 + 2R_e + R_i)}$$

where E_s is voltage gradient in the sea; l_s is the distance between marine electrodes, 400 m; l_t is the distance between the tank electrodes, 1 m; R_1 is water resistance in the tank, 45 Ω ; R_i is the overall resistance of electric line (seawater resistance + marine electrodes resistances + cable resistances), 100 Ω ; R_e is the

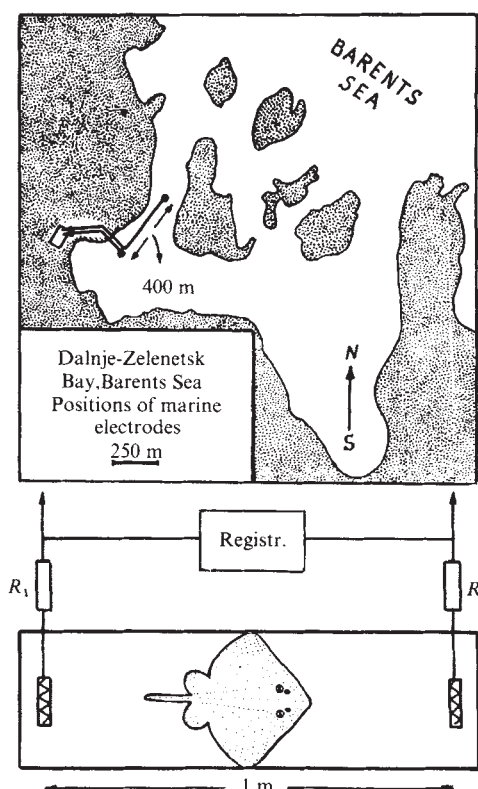


Fig. 1 Experimental set-up. For details see the text.

resistance of an electrode in the tank, $30\ \Omega$; $R_1 = R_2$ are resistances in series.

To make E_i equal to E_s , R_1 and R_2 should be

$$R_1 = R_2 = 1/2[(l_s R_i / l_i) - R_1 - 2R_e - R_i] \approx 9\ \text{k}\Omega$$

To estimate the electroreceptor unit response to natural stimulus, spike frequency was compared to the magnitude of the voltage gradients produced by telluric currents. Magnetic field variations were registered.

Measurements were made during a magnetic substorm which appeared together with the polar light on 25–26 February, 1978. The rate of change in the geomagnetic vertical component at the time when measurements were made was 0.2 to $1.0\ \text{nT s}^{-1}$ (2×10^{-6} – $10^{-5}\ \text{G s}^{-1}$); maximal deviation from the background field was $216\ \text{nT}$. Short-period (1–3 min) oscillations of telluric voltage gradients were observed to be synchronous with variations of the geomagnetic field. Voltage gradients in the sea (and, consequently, in the tank) were from $\pm 0.6\ \mu\text{V cm}^{-1}$ to (at times) $\pm 1.25\ \mu\text{V cm}^{-1}$.

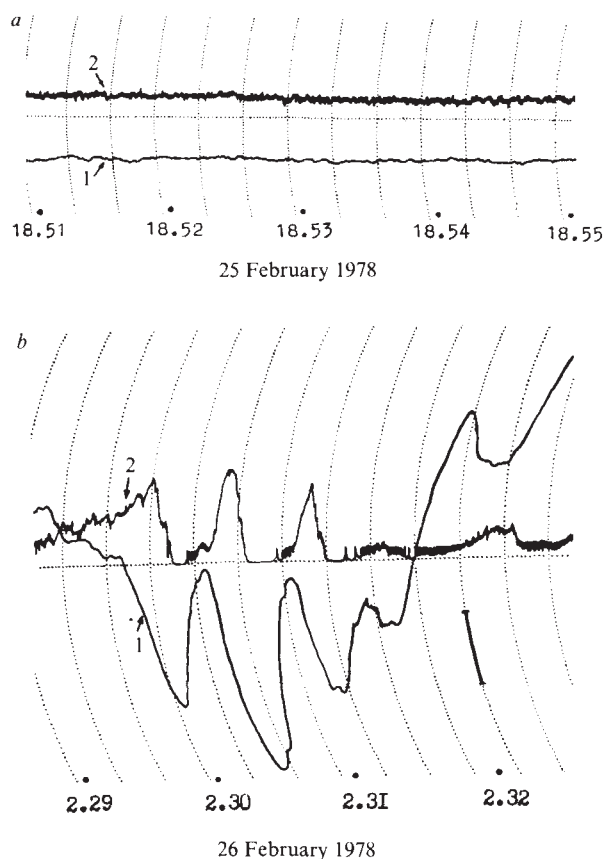


Fig. 2 Synchronous recording of induced electric field in the sea (1) and the time course of spike frequency of a single electroreceptor (2) before (a) and during the magnetic substorm (b). Vertical bar: $0.2\ \mu\text{V cm}^{-1}$; $15\ \text{imp s}^{-1}$ (GMT).

Figure 2 shows that electric field oscillations due to geomagnetic variations produced a clear-cut response in the electroreceptors, as judged by corresponding changes in spike frequency. The spike frequency was sensitive not so much to the absolute value of the electric field as to its rate of change, that is, the field derivative with respect to time. Such a response may be due to adaptation of the receptor to a prolonged stimulus. Receptor sensitivity was sufficient to detect a change in the electric field as small as $0.006\ \mu\text{V cm}^{-1}\ \text{s}^{-1}$.

These measurements clearly indicate that the ampullae of Lorenzini can detect the electric field induced by variations of the Earth's magnetic field. This capacity is likely to be more strongly expressed within the coastal area, and this could be the result of an enhancement of telluric currents near the coast due

to 'coast effect' (ref. 8). Such a suggestion agrees with experimental data indicating that the electroreceptor response associated with changes in magnetic field is maximal when a fish is near the wall of a tank⁶. The marine electrodes in our experiments were placed in a bay screened from the open sea by islands which reduced the coast effect of telluric currents⁹. Otherwise, the effects observed might have been greater.

Further studies should provide information about the biological role and practical applications of the detection of telluric currents by fish.

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Correlation between heterozygosity and subunit molecular weight

GENIC heterozygosity at enzyme loci has been examined for correlation with parameters as diverse as substrate specificity¹, physiological function² and quaternary structure³. However, none of these has provided an adequate explanation for allozymic variation in general. One report⁴ suggested a positive relationship for *Drosophila* enzymes between heterozygosity and subunit molecular weight (MW), which can be equated to the size of the structural gene element. Various models, both neutral and selective, would predict a correlation between gene size and variability^{5–7}. Recently, a similar correlation has been found within three classes of vertebrates^{8,31}, and between the number of rare alleles and subunit size in human populations^{9,10}. We report here that we have tested the relationship between heterozygosity and subunit size in *Drosophila* species with a new selection of enzymes. Our results indicate that the correlation is indeed general. The relationship is quasi-linear and does not differ significantly from several models. The results support the hypothesis that there are constraints on the number of sites available to charge substitution within an enzyme molecule.

For our test we used data on six of the enzymes used by Koehn and Eanes⁴ and estimated the MWs on a series of at least seven enzymes in *Drosophila melanogaster* for which such information was not previously available. We excluded several enzymes from our analysis because of inadequacies in the data. No esterases or phosphatases were used as their homology across *Drosophila*

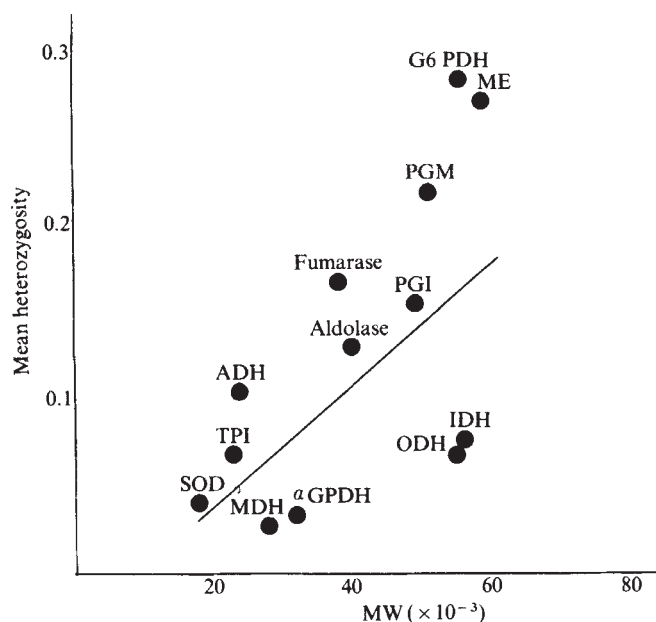


Fig. 1 Mean heterozygosity as a function of MW. SOD, 18,000; TPI, 23,000; ADH, 24,000; MDH, 28,000; α GPDH, 32,000; fumarase, 38,000; aldolase, 40,000; PGI, 49,000; PGM, 51,000; ODH, 55,000; G6PDH, 55,000; IDH, 56,000; ME, 58,000. Each point represents the mean heterozygosity value for an enzyme over all species surveyed for that enzyme. The regression line indicated ($y = 0.0000353x - 0.0289$) was calculated from the full data set of 118 observations (which is available on request).

species is not clear. In addition to these restrictions on the enzymes used we have made a new selection of population data. In so doing, we only used data from those sources which permitted calculation of the actual numbers of each allele observed. However, whereas Koehn and Eanes⁴ did not consider samples where an enzyme was completely monomorphic, we included all such samples.

The sedimentation constants ($s_{20,w}$) of phosphoglucose isomerase (PGI) and triosephosphate isomerase (TPI) were estimated from sucrose density gradients¹¹, relative to *D. melanogaster* alcohol dehydrogenase (ADH) and rabbit muscle lactate dehydrogenase (LDH). The means and standard errors of six estimates were: PGI, 6.6 ± 0.23 ; TPI, 4.5 ± 0.13 . Stokes' radius estimates of 35.5 Å and 28.6 Å, respectively, were obtained from gel filtration relative to three standards¹². From these data, the native MWs of these enzymes were calculated to be 97,000 for PGI, and 47,000 for TPI, assuming a partial specific volume of $0.725 \text{ cm}^3 \text{ g}^{-1}$.

The MWs of enzymes with too little activity for convenient assay in the sucrose density gradients were estimated directly from gel filtration chromatography¹³. Relative to five standards, the native MWs of fumarase, isocitrate dehydrogenase (IDH) and phosphoglucomutase (PGM) were estimated as 150,000, 112,000 and 51,000, respectively. Full data on the MW determinations of these and other *Drosophila* enzymes will be published elsewhere.

The enzymes used in investigating the relationship between heterozygosity and subunit size were: TPI, PGI, IDH, fumarase and PGM from above; malic enzyme (ME) and glucose 6-phosphate dehydrogenase (G6PDH) for which direct estimations of subunit size (58,000 and 55,000) have been made in our laboratory from purified enzyme¹⁴, and, from Koehn and Eanes' analysis⁴, superoxide dismutase (SOD), malate dehydrogenase (MDH), α -glycerophosphate dehydrogenase (α GPDH), octanol dehydrogenase (ODH), aldolase and ADH. The structures of the new enzymes have been established mainly by starch-gel electrophoresis. Other work in progress in our laboratory has necessitated the collection of variants at all these loci in *D. melanogaster*. From this work, TPI, PGI and IDH have

been deduced to be dimers from the appearance of three clear bands in heterozygotes, whereas PGM is a monomer and fumarase and aldolase are tetramers (R. A. Voelker and C.H.L., unpublished data). For the remaining enzymes, the structure given by Koehn and Eanes⁴ was assumed.

The population data used in the analysis were carefully selected, as has been described. (They were taken from refs 15–25 and from unpublished data of F. J. Ayala.) Where more than one suitable sample was available for a species, the data were pooled so that each species was given equal weight. Data from a total of 15 species were used in the analysis. The correlation analysis was carried out on the new enzyme set, on the set used by Koehn and Eanes, and on both combined. A non-parametric test for rank correlation was made using Kendall's τ , as neither variable is normally distributed, and the results are given in Table 1. It can be seen that there is a highly significant correlation between heterozygosity and subunit MW in the combined data set. Figure 1 shows the distribution of the data and a least-squares regression line which was fitted to the whole data set. For clarity, only one point is shown for each enzyme, the mean value taken over all species with data for that enzyme.

We have been able to confirm the conclusion of Koehn and Eanes⁴ that there is a positive correlation between allozymic heterozygosity and subunit MW. Having made a stringent selection of the data and used a test of reduced power we are satisfied that this relationship is general. Nei *et al.*¹⁰ have recently examined the relationship between MW and heterozygosity in vertebrates and obtained a significant correlation on data from three classes. These authors claimed that the magnitude of their correlation was "roughly" in agreement with the predictions of a neutral model. We have examined our data in a similar way and have also found agreement between our correlation and the predictions of both the infinite alleles⁵ and the charge-state models²⁶. However, the regression line has a wide confidence belt and almost any model which predicts a positive monotonic relationship between the two variables would also fit our data. Two alternative such models are those of Gillespie⁶ and Watterson⁷. In Gillespie's random-environments model the heterozygosity at a locus depends inversely on the relative effect of a substitution on enzyme function. In general, the larger an enzyme molecule, the smaller would be the relative effect of a mutation. In Watterson's stochastic heterotic model heterozygosity is as predicted by the infinite-alleles model when $\sigma = 2N_e s = 0$. We found that although the predictions diverge as σ increases, for $\sigma = 0(1)$ this model does not differ greatly from the neutral case in the region of heterozygosity values of our data.

We have also carried out a two-way analysis of variance on our heterozygosity data by gene and species, and the results

Table 1 Correlation between allozymic heterozygosity and subunit MW

Data set	Kendall's τ	Probability of a higher τ value
(1) TPI, PGI, IDH, PGM, ME, fumarase, G6PDH	0.159	0.13
(2) ADH, Aldolase, SOD, MDH, ODH, α GPDH	0.102	0.26
(3) All data—(1)+(2)	0.213	0.0013

The MWs of TPI and PGI were determined by sucrose density gradient sedimentation and those of fumarase, IDH and PGM by gel filtration on Sephacryl S-200. The structure of these enzymes was determined by starch-gel electrophoresis as described in the text. Subunit sizes were thereby assigned as follows: TPI, 23,000; PGI, 49,000; fumarase, 38,000; IDH, 56,000; PGM, 51,000. Subunit sizes for ME (58,000) and G6PDH (55,000) have been determined from purified enzyme. Data set (1) consists of those enzymes whose MW was not previously known. Data set (2) consists of enzymes previously used in the analysis of Koehn and Eanes⁴. In each case new population data were selected as indicated in the text. Note that G6PDH was used in Koehn and Eanes' analysis⁴. It is included in data set (1) because a new MW determination is available which represents a substantial change from the value previously used.

Table 2 Analysis of variance of heterozygosity by gene, species and MW

	Sum of squares	Degrees of freedom	Variance component per observation
Species	0.4792*	14	0.0041
Gene	0.6365†	12	0.0055
MW	0.2328		0.0020
Other gene effects	0.4037		0.0035
Error	1.7973	91	0.0153
Total	2.913	117	0.0249

The analysis of variance was first carried out by gene and species on the full data set. The component of the variance due to gene effects was then partitioned into that due to MW and that due to other gene-specific effects as described in the text. The level of significance of the correlation with MW is given in Table 1.

*Significant at 5% level.

†Significant at 1% level.

(Table 2) show significant effects due to both. We have calculated the correlation coefficient between heterozygosity and MW, corrected for species effects ($r = 0.309$; $P < 0.01$). Following Nei *et al.*¹⁰, r^2 is the ratio of the co-variance between gene-average heterozygosity and MW to the product of the variance in MW and the total variance in heterozygosity less that due to species. We were thus able to divide the genic component of the variance into that due to MW and that due to other gene effects. By partitioning the variance in heterozygosity in this way, we have been able to compare the component due to MW with that due to species. We find (Table 2) that the variance component due to MW is about half that due to species.

In the infinite-alleles model of Kimura and Crow⁷, heterozygosity is determined by the product of effective population size and mutation rate. Assuming that the latter is proportional to structural gene size, it is interesting that the variance component of our data that is due to MW is so large relative to the species effects, as there is only a threefold range in MW. This would suggest that in equilibrium conditions the range in total population size, being the main species effect under this model, would be of the order of sixfold. We consider this a challenge to the infinite-alleles model. Although one possible interpretation could be that the effective population number of these 15 *Drosophila* species only varies to a similar degree, this is unlikely as the sample includes both equatorial forest species (for example, *D. willistoni*) and cactophilic species (*D. arizonensis*). It could also be argued that they have not attained equilibrium due to fluctuations of population size, but it seems more plausible to us that there are general constraints on the number of sites in a gene where a charge substitution is 'evolutionarily acceptable'. This alternative has been suggested by the analysis of the evolution of amino acid sequence in cytochrome c, fibrinopeptide A and haemoglobin²⁷⁻²⁹. Werhahn and Gulizia³⁰ have pointed out that the effect of population size on equilibrium heterozygosity decreases with the number of mutable sites. Thus, the infinite-alleles model is not consistent with all aspects of our data, and although it could be said to predict a correlation between heterozygosity and MW, so could several other models.

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Acetylcholine-elicited responses in primary and secondary mammalian oocytes disappear after fertilisation

THE recent finding¹ of acetylcholine (ACh) receptors on the amphibian oocyte membrane raises two main questions: (1) is the presence of ACh receptors a feature common to all vertebrates including mammals, and (2) does the sensitivity to ACh undergo developmental changes throughout oocyte growth and maturation, or following fertilisation? We have investigated these problems using conventional electrophysiological techniques, and show here that depolarising and/or hyperpolarising potentials are elicited by ACh in mouse dictyate and metaphase II oocytes, but not after fertilisation.

Fully grown dictyate mouse oocytes from antral follicles (see legend to Fig. 1 for technical details) had a resting membrane potential ranging from -20 to -40 mV (-27.1 ± 1.1, mean ± s.e.m., $n = 57$). When these cells were perfused with a medium containing 1×10^{-4} to 2×10^{-2} M ACh, a dose-dependent membrane depolarisation usually occurred (Figs 1, 2a). The ACh-elicited depolarisation took many seconds to develop, as previously shown in *Xenopus* oocytes¹, and the delay between ACh application and the onset of the response was never shorter than 15-20 s. Such a delay was not significantly modified by increasing the bath temperature to 37 °C. At lower ACh concentrations (1×10^{-8} – 1×10^{-5} M), the response to ACh differed among the oocytes studied. Some oocytes underwent membrane hyperpolarisation after an initial depolarisation, whereas others displayed only the hyperpolarising response, or no resting potential change at all. The hyperpolarising response was only occasionally observed at high ACh doses (up to 2 mM) (Fig. 2b). Both depolarising and hyperpolarising ACh potentials gradually 'desensitised' during maintained application of the drug (Fig. 2a, b), and preliminary exposure of oocytes to ACh concentrations as low as 10^{-11} M completely prevented all membrane responses to maximal ACh doses (up to 2×10^{-2} M). The ACh-elicited depolarisation was associated with a significant decrease in membrane input resistance (see legend to Fig. 1 for technical details), whereas the hyperpolarisation was accompanied by a significant increase in membrane input resistance. Ionophoretic application of ACh on to oocyte membrane occasionally elicited very small depolarising or hyperpolarising responses in antral oocytes, and here again these responses took many seconds to develop, as previously shown in *Xenopus* oocytes¹.

Incubation of antral mouse oocytes in Ca^{2+} -free (Ca^{2+} replaced by Mg^{2+}), Na^{+} -free (Na^{+} replaced by choline), or K^{+} -free (K^{+} replaced by Na^{+}) media did not seem to modify the ACh-elicited depolarisation. However, in the presence of Cl^{-} -free medium (Cl^{-} replaced by SO_4^{2-} or propionate), the ACh depolarising response was enhanced, although a maintained incubation of the cell in Cl^{-} -free medium completely prevented the depolarising response. These findings apparently indicate that chloride ions are involved in the ACh-elicited depolarisation. Similar conclusions were drawn in *Xenopus* oocytes¹. Similarity between amphibian and mammalian oocyte ACh receptors is further indicated by the ineffectiveness of (+)tubocurarine (up to 10^{-4} M) in blocking responses in antral mouse oocytes, and by the effectiveness of atropine (10^{-7} – 10^{-6} M) in preventing ACh membrane depolarisation. Atropine did not seem to abolish the ACh-elicited hyperpolarisation.

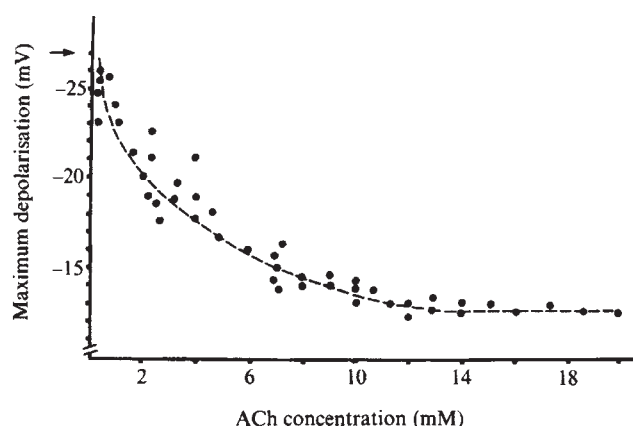


Fig. 1 Depolarising response of antral mouse oocytes in the presence of increasing ACh concentrations. The maximum depolarisation level after ACh perfusion obtained in 47 independent determinations is plotted against the corresponding ACh concentration in the perfusion bath. The arrow indicates the mean membrane resting potential of the tested cells. Swiss CD1-COBS mice from Charles River Italia were used in all experiments. Dictyate oocytes at an intermediate stage of growth (vitellus diameter 60–65 μm , pre-antral, from follicle stages 5a and 5b, ref. 2) and at the end of growth (vitellus diameter 75–85 μm , antral, from antral follicles) were obtained mechanically, by pricking infant (pre-antral) and adult (antral) ovaries with a needle, and were completely freed from surrounding granulosa cells by gentle pipetting in phosphate-buffered saline (PBS) supplemented with pyruvate, lactate and bovine serum albumin. Metaphase II oocytes and fertilised eggs were obtained from mice treated with pregnant mare serum and human chorionic gonadotropin, and isolated from cumulus cells by brief exposure to hyaluronidase in PBS. Following isolation, oocytes and eggs were carefully washed in the complete bath medium of Okamoto *et al.*³ and immediately processed for electrophysiology. Experiments were routinely carried out at room temperature (23–25 °C) in a 1 ml perfusion bath. Similar results were obtained from control experiments carried out at 37 °C. Glass microelectrodes filled with 1.5 M K acetate (resistance 15–30 M Ω) were used in all experiments. Determinations of membrane resistance were made with the aid of a modified bridge circuit^{4,5} by a series of brief hyperpolarising pulses of a few mV amplitude.

To investigate whether ACh sensitivity changes throughout oocyte growth, we have examined pre-antral oocytes at an intermediate stage of cell growth. A mean resting potential of -39.3 ± 4.3 mV ($n = 12$) was found in pre-antral oocytes. These cells responded to both high and low ACh doses mainly by membrane hyperpolarisation accompanied by an increase in input membrane resistance. Few cells underwent ACh depolarisation up to -25 and -30 mV (10^{-4} – 10^{-2} M ACh), and here a decrease in membrane resistance was observed. Taken together, these findings indicate that ACh sensitivity is a

constant feature of dictyate mouse oocytes, at least from intermediate to terminal stages of cell growth. The depolarising ACh response apparently reached a maximum at the end of the cell growth period, that is, with the appearance of the antrum inside the follicle.

In metaphase II oocytes, a mean resting potential of -24.3 ± 1.4 mV ($n = 12$) was observed, in agreement with previous observations³. These oocytes responded to ACh with a pattern of membrane potential depolarisation and/or hyperpolarisation similar to that observed in the antral cells.

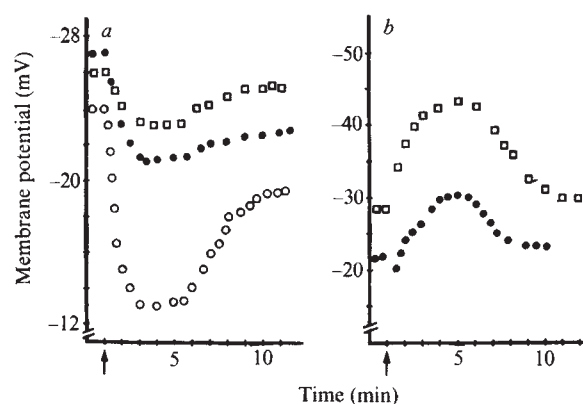


Fig. 2 ACh-elicited depolarisation (a) and hyperpolarisation (b) in five antral mouse oocytes treated with different doses of ACh. Following ACh application (arrow), a depolarising or hyperpolarising response gradually developed, and the maximum level of response was attained after 2–4 min. Note desensitisation after prolonged exposure of the cells to the drug. a: $\square$, 0.5 mM; $\bullet$, 2 mM; $\circ$, 7 mM. b: $\square$, 10^{-7} M; $\bullet$, 2 mM.

Fertilised eggs had a membrane potential (-41.8 ± 3.1 , $n = 34$) higher than that of unfertilised oocytes, in agreement with previous observations⁶. Unlike primary and secondary oocytes, fertilised mouse eggs displayed no ACh-elicited responses. ACh doses ranging from 10^{-8} M up to 1.5×10^{-2} M did not elicit any change in membrane potential and input membrane resistance in eggs collected at various times after fertilisation (5–20 h). We have also tested two-cell embryos (membrane resting potential -48.4 ± 2.2 mV, $n = 6$), and here again we did not observe any ACh response. Therefore, the disappearance of cholinergic response is an early event following fertilisation, lasting at least until the two-cell stage of embryonic development. It would be interesting to know whether this disappearance of oocyte ACh sensitivity following fertilisation is directly related to sperm penetration. We favour this hypothesis, as preliminary experiments have shown that responses to ACh are still present in both spontaneously activating and Sr^{2+} -activated mouse eggs (see ref. 7 for parthenogenetic activation of mouse eggs).

We conclude that ACh sensitivity is probably a general feature of vertebrate oocytes, even if in the mouse it is lower than in the amphibians¹. Mouse oocyte sensitivity to ACh undergoes developmental changes throughout oocyte growth, and it is not grossly affected by the maturation process at ovulation. Finally, ACh sensitivity is a property peculiar to primary and secondary oocytes, as it is lost following fertilisation. This suggests that ACh receptors in vertebrate oocytes may have some specific role in the maturation and/or fertilisation processes.

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Hypothalamic enkephalin neurones may regulate the neurohypophysis

WE have previously reported that significant amounts of immunoreactive (ir)-Leu⁵-enkephalin were present in extracts of the neurointermediate lobe of the rat pituitary¹. Negligible amounts of the pentapeptide were detected in the anterior lobe. In these assays, the concentration of Leu⁵-enkephalin in the neurointermediate lobe was higher than in the globus pallidus, the brain region reported to contain the densest enkephalinergic innervation². The high content of (ir)-Leu-enkephalin in the neurointermediate lobe of the pituitary led us to further investigation of its distribution and possible function. We report here that (ir)-enkephalins in the pituitary are concentrated in nerve fibres projecting from the hypothalamus to the pars nervosa and that this pathway may be involved in the regulation of neurohypophyseal neurosecretion.

Under a dissecting microscope the rat pituitary could be easily separated into the anterior lobe (pars distalis) and the neurointermediate lobe, and the latter dissected into pars nervosa and the lobules of the pars intermedia. The isolated elements of single pituitaries were each then homogenised and assayed by radioimmunoassay^{1,3} for β -endorphin, and Leu⁵-enkephalin (Fig. 1). The major portion of (ir)-Leu⁵-enkephalin (76%) was

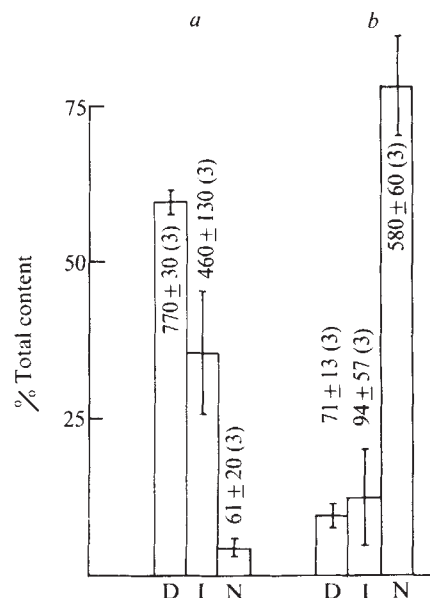


Fig. 1 Pituitaries of Sprague-Dawley male rats (200 g) were dissected under the microscope and pars distalis (D), pars intermedia (I) and pars nervosa (N) were extracted in hot 1 M acetic acid as described in ref. 3, which also contains the complete procedure for the radioimmunoassays of β -endorphin and Leu⁵-enkephalin. Dose-response curves of the pituitary extract shows parallelism with synthetic Leu⁵-enkephalin. Gel filtration of the extracts on a Bio-Gel P-60 column (50 $\times$ 0.7 cm) equilibrated in 50% acetic acid showed that all the (ir)-Leu⁵-enkephalin was eluted in a symmetrical peak indistinguishable from that obtained with synthetic Leu⁵-enkephalin. Neither vasopressin (AVP or LVP) nor oxytocin crossreact in our Leu⁵-enkephalin radioimmunoassay at doses up to 100 g per assay tube. *a*, β -endorphin (ng per pars); *b*, Leu⁵-enkephalin (pg per pars).

found in the pars nervosa. In using a radioimmunoassay for Met⁵-enkephalin described elsewhere⁴, we found that the pars nervosa contained 1,920 $\pm$ 240 pg (n = 5; mean $\pm$ s.e.m.) of (ir)-Met⁵-enkephalin. The ratio of (ir)-Met⁵-enkephalin to (ir)-Leu⁵-enkephalin was $\sim$ 3, a similar value to that found in other rat brain areas rich in enkephalins⁴. (ir)- β -endorphin was almost absent from the pars nervosa, but was present in both pars distalis and pars intermedia, as indicated in previous studies^{1,5}.

Using a peroxidase-coupled goat anti-rabbit IgG for indirect immunocytochemistry⁶, fine varicose nerve fibres showing Leu⁵-enkephalin immunoreactivity were localised in the pars nervosa. These fibres were concentrated on the perimeter of the neural lobe and more or less absent from the centre of the tissue (Fig. 2*b*). Fibres with similar immunoreactivity could be traced in favourable sections through the pars tuberalis (pituitary stalk), and as reported by others, were also present in supraoptic and paraventricular nuclei (ref. 7 and Fig. 2*a*).

The antiserum also stained corticotroph cells of the anterior lobe and all cells of the pars intermedia. As these intermediate lobe cells contain high amounts of β -endorphin, postmortem degradation by peptidases could have given rise to Met⁵-enkephalin in sufficient amounts to be detected by our enkephalin antiserum.

After pretreatment with colchicine, (ir)-Leu⁵-enkephalin cell bodies were visualised in the paraventricular (Fig. 2*a*) and supraoptic nuclei of the hypothalamus. Varicose fibres from these neurones could be traced through the lateral hypothalamic area, along the course followed by magnocellular neurosecretory axons. Lesioning experiments further suggest that the enkephalinergic cell bodies in the magnocellular nuclei have projections in the pars nervosa. Six days after sectioning the pituitary stalk or lesioning the medial basal hypothalamus, (ir)-enkephalinergic fibres were absent from the pars nervosa.

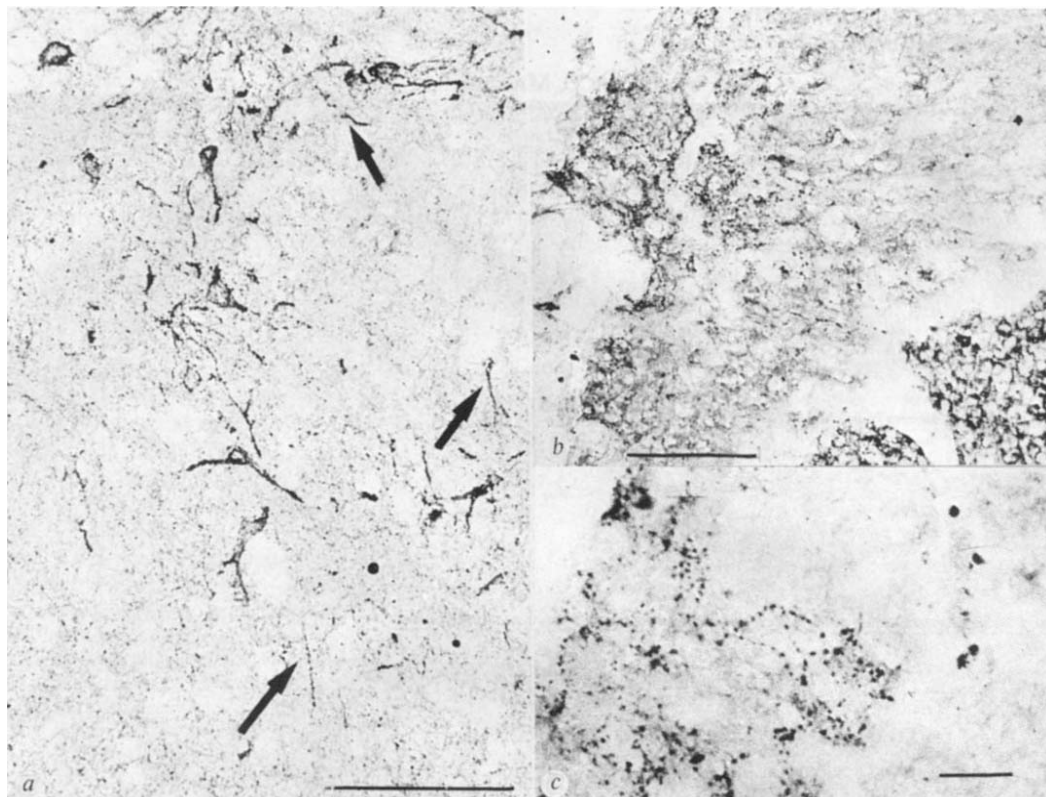
Table 1 (ir)-Leu⁵-enkephalin in neurointermediate lobe after hypothalamic lesions

	pg per lobe (mean $\pm$ s.e.m.)
Controls (6)	503 $\pm$ 31
Paraventricular nucleus lesions (8)	289 $\pm$ 21*
Paraventricular and supraoptic lesions (2)	55

Discrete anodal lesions were made with stainless steel wires, insulated to the tips and stereotactically positioned in the paraventricular nuclei or in both paraventricular and supraoptic nuclei of male, Sprague-Dawley rats (250 g). Control lesions were placed in the cortex. Seven days after lesioning, radioimmunoassay for Leu⁵-enkephalin was carried out on neurointermediate lobes extracted in 1 M acetic acid as described in Fig. 1. All lesion sites were histologically verified. Numbers in parentheses refer to the number of animals tested.

* P < 0.001, t test.

Fig. 2 Photomicrographs of rat hypothalamus (*a*), and neurohypophysis (*b*, *c*) reacted with rabbit anti-Leu⁵-enkephalin and a horseradish peroxidase-tagged goat anti-rabbit IgG, as described elsewhere⁹. *a*, After intracerebroventricular colchicine (25 µg, 24 h) neurones in the lateral pole of the paraventricular nucleus exhibit positive immunoreactivity in their dendrites, perikarya and in some finer varicose processes (arrow) presumed to be axons. *b*, Neurohypophysis and intermediate lobe (lower right) of normal rat. Fine varicose immunoreactive fibres can be seen only at the perimeter of the neurohypophysis. Cells of the intermediate lobe show general cytoplasmic immunoreactivity. *c*, Higher magnification view of immunoreactive fibres at the perimeter of the neurohypophysis. Fibres identical to these were seen in occasional patches in Brattleboro rats pretreated with colchicine (24 h, 25 µg), but in untreated Brattleboro rats, no neurohypophysial immunoreactivity was observed. Note that at this higher magnification, the immunoreactive fibres seem to be enveloping groups of non-reactive axons at the perimeter of the neurohypophysis. Calibration bars: *a*, 50 µm; *b*, 25 µm; *c*, 10 µm.



As shown in Table 1, radioimmunoassay measurements confirmed that discrete damage to the paraventricular nucleus resulted in a 40% decrease in the levels of (ir)-Leu⁵-enkephalin in neurointermediate lobe extracts. Further damage to the supraoptic nucleus reduced levels to 10% of controls.

The detection of a neuronal pathway between enkephalin-immunoreactive neurones in the magnocellular hypothalamic nuclei and enkephalin-(ir) terminals in the pars nervosa suggests at least two possible functional roles. As with other neurohypophysial hormones, enkephalin could be released as a neurohormone into the bloodstream with vasopressin or oxytocin. Alternatively, enkephalinergic fibres could somehow interact with the secretion of neurohypophysial hormones. Indeed, we find that salt loading decreases (ir)-Leu⁵-enkephalin in the neurointermediate lobe (Table 2). Five days after substitution of 2% NaCl for drinking fluid, the vasopressin stores in the neurohypophysis decrease to ~20% of control⁸. In these conditions, we find that (ir)-Leu⁵-enkephalin content drops to 37% of control. However, this degree of dehydration did not affect β -endorphin levels in the anterior and intermediate lobes of the pituitary (Table 2). (ir)-Leu⁵-enkephalin levels were also not changed in the hypothalamus during this treatment.

Levels of thyrotropin-releasing hormone (TRH) and somatostatin in these extracts were also determined, using radioimmunoassays described elsewhere^{9,10}. These two peptides are highly concentrated in the hypothalamus and also in the neurointermediate lobe of the pituitary (ref. 19 and W. Vale, personal communication). In the neurointermediate lobe we found 120 ± 16 pg of (ir)-TRH per lobe ($n=6$) and 810 ± 220 pg of (ir)-somatostatin per lobe ($n=6$). Neither pituitary nor hypothalamic levels of TRH and somatostatin were affected by dehydration, indicating that the mechanisms producing the (ir)-Leu⁵-enkephalin decrease in the neurohypophysis are peptide specific.

The striking effect of dehydration on the (ir)-Leu-enkephalin content on the neurointermediate lobe led us to examine the

distribution of enkephalin in the Brattleboro rat. In this strain the homozygous offspring are polyuric and totally unable to synthesise or secrete vasopressin⁸. When compared with unaffected heterozygote littermate controls, the (ir)-Leu⁵-enkephalin levels (Table 2) in the neurointermediate lobe of Brattleboro rats are very low (33% of the heterozygous controls). No differences between homozygous and heterozygous Brattleboros were observed in the (ir)-Leu⁵-enkephalin

Table 2 Opioid peptides in pituitary after dehydration

Rat strain	(ir)- β -endorphin		(ir)-Leu ⁵ -enkephalin
	ng per lobe (mean $\pm$ s.e.m.)		pg per lobe (mean $\pm$ s.e.m.)
	Anterior	Neuro-intermediate	Neuro-intermediate
Sprague-Dawley			
Controls (6)	375 $\pm$ 46	661 $\pm$ 66	880 $\pm$ 60
5 d with 2% NaCl drinking fluid (6)	475 $\pm$ 79	647 $\pm$ 117	320 $\pm$ 80†
Brattleboro			
Controls (<i>Di</i> /?) (4)	850 $\pm$ 103	705 $\pm$ 80	470 $\pm$ 19
Polydyspic (<i>Di</i> / <i>Di</i>) (4)	1,205 $\pm$ 98*	1,140 $\pm$ 140*	156 $\pm$ 26†

In two separate experiments, Sprague-Dawley male (200 g) rats which had received 2% NaCl as drinking fluid for 5 d were compared with their controls which had received plain tap water, and male (120 g) homozygous (*Di*/*Di*) Brattleboro rats were compared with their control littermates (*Di*/?). Radioimmunoassay for β -endorphin and Leu⁵-enkephalin was carried out on 1 M acetic acid extracts as described in Fig. 1. Weights of the control Sprague-Dawley anterior and neurointermediate lobe were, respectively, 6.2 ± 0.3 and 0.95 ± 0.07 mg. The 2% NaCl animals had a statistically significantly ($P < 0.01$) decreased weight of their anterior lobe (4.2 ± 0.4) and no statistically significant change in their neurointermediate lobe weight (0.80 ± 0.04).

* $P < 0.05$; † $P < 0.01$, *t* test.

levels of hypothalamus or striatum. (ir)- β -endorphin was slightly elevated in the homozygous Brattleboro rats, both in the anterior and posterior lobes of the pituitary, but was unchanged in the hypothalamus. Immunocytochemical studies of the enkephalin fibres in the pars nervosa of the Brattleboro indicated that the number of enkephalin fibres here are decreased and their distribution and morphology somewhat dystrophic (Fig. 2c).

Morphine injections are known to release vasopressin into the bloodstream¹¹, and opiate receptors are also concentrated in the pars nervosa¹². Therefore, we propose that these receptors are related to the enkephalinergic innervation of the pars nervosa. Furthermore, because dehydration decreased both the enkephalin and the vasopressin content of the pars nervosa, we propose that pituitary enkephalin fibres may have a role in the regulation of vasopressin or other magnocellular hormonal secretion.

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The granulated peripolar epithelial cell: a potential secretory component of the renal juxtaglomerular complex

THE renal juxtaglomerular complex is conventionally considered to consist of the afferent and efferent glomerular arterioles, the macula densa of the distal tubule, and the interposed polar cushion region¹. The afferent arteriolar wall contains myoepithelial cells characterised by the presence of multiple secretory-type cytoplasmic granules. It is widely accepted that such granules contain renin², the hormone responsible for the production of angiotensin I from plasma renin substrate. We now report the finding of another potential secretory component of the juxtaglomerular complex, raising the possibility of unsuspected functional interactions in this region.

During a study of the influence of salt-balance status on juxtaglomerular morphology in sheep, considerable difficulty

was experienced in detecting arteriolar myoepithelial cell granules using Bowie's stain³ or electron microscopy. However, sections through the vascular pole of many glomeruli revealed the presence of a new, distinctive type of cell which contains multiple cytoplasmic granules closely resembling those of the arteriolar myoepithelial cells. These new cells are in an epithelial position, encircling the origin or polar region of the glomerular tuft (Fig. 1). We have therefore called them 'peripolar cells'. They form junctional complexes with the parietal epithelium of Bowman's capsule on one side and visceral podocytic epithelium on the other; one surface is attached to the basement membrane of Bowman's capsule and the other is directly exposed to the urinary space. Up to four such peripolar cells have been observed surrounding the origin of any one glomerular tuft. The chief morphological characteristic of such cells is the presence of large numbers of closely packed cytoplasmic granules which react positively with methylene blue, the periodic acid-Schiff technique, Brilliant Crystal Scarlet (using Lendrum's Martius-Scarlet Blue stain⁴ following fixation in Helly's fluid) and Bowie's stain. Electron microscopically, the granules are membrane-bound, mostly round in shape and contain homogeneous, finely fibrillo-granular electron-dense material (Fig. 2). Typically, most granules range from 100 to 500 nm in diameter. Although most granules in any one cell are of fairly uniform electron density, a few granules display a paler matrix. Granules at the cell periphery have occasionally been observed in the process of discharging their contents into the urinary space. The cytoplasm also contains mitochondria, prominent Golgi membranes, segments of rough endoplasmic reticulum, free ribosomes, and scattered microfilaments and microtubules.

Granulated peripolar cells are usually large in sheep, are sometimes prominent in man, but are less readily found and much smaller in rats and mice. It is most interesting that, thus far, prominent peripolar cells have been found in species with poorly developed afferent arteriolar granulation; in contrast, peripolar cells seem to be relatively smaller in species with abundant arteriolar granulation. The composition of the granules and their function await elucidation. In view of their topographical location, it is almost certain that they participate in juxtaglomerular complex activity. They are ideally situated to release factors directly into Bowman's capsular space, perhaps triggered by electrolyte changes in the glomerular ultrafiltrate, or by variations in afferent or efferent arteriolar calibre (leading to alterations in the diameter of the polar region of the glomerular tuft), or in response to diffusible mediators released from other components of the complex. Factors released from

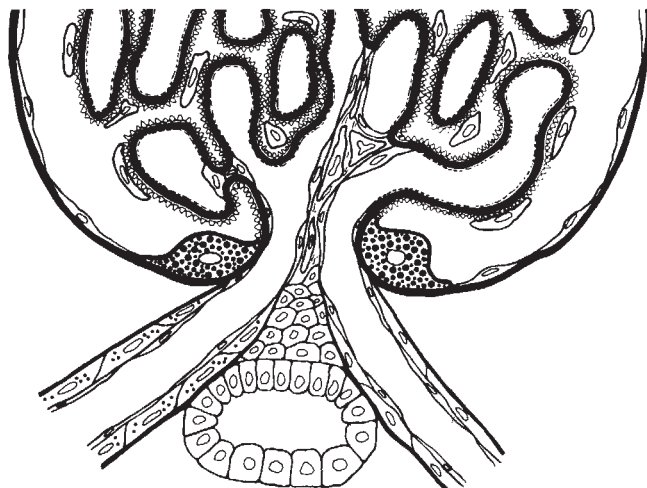


Fig. 1 Schematic drawing of sheep juxtaglomerular region showing two granulated 'peripolar cells' on each side of origin of glomerular tuft at junction between parietal and podocytic epithelium. Note afferent arteriole containing relatively few myoepithelial cell granules (lower left), efferent arteriole (lower right), macula densa of distal tubule (lower central) and polar cushion cells interposed between macula densa and origin of glomerular tuft.

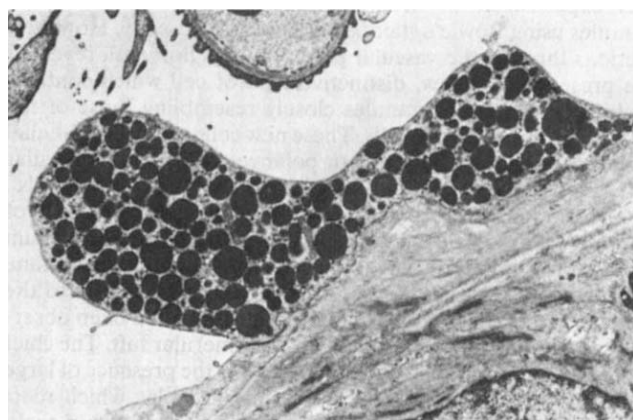


Fig. 2 Electron micrograph of sheep 'peripolar cell' containing multiple electron-dense cytoplasmic granules; its free surface is directly exposed to Bowman's capsular space. Note portion of juxtaglomerular arteriole (lower right) and glomerular tuft capillary (upper central). $\times 5,000$.

peripolar cells could pass into the tubular fluid and be involved in the modulation of secretory or resorptive functions of tubular cells. There has been much study directed towards determining the factors controlling proximal tubular sodium reabsorption and glomerulotubular balance⁵⁻⁷. One school of thought holds that a previously unrecognised hormone is involved in these processes. It has recently been reported that proximal tubular fluid contains a factor which mediates increased volume reabsorption in the proximal tubule in response to increased glomerular filtration rate⁸; it was suggested that such increased volume reabsorption results from increased proximal tubular sodium reabsorption⁸. The proximal tubular fluid factor responsible for these effects was not present in an artificial ultrafiltrate of plasma⁸, indicating that it is added to the tubular fluid after filtration of plasma across the glomerular capillary wall. It is possible that the peripolar cell is the source of such a factor, thereby having a key role in glomerulotubular balance and electrolyte homeostasis.

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T-cell hybrids bear Fc γ receptors and secrete suppressor immunoglobulin binding factor

PROGRESS in the study of antibody structure and function came from the establishment of plasmacytomas and B-cell hybrids¹ which produce large amounts of monoclonal antibodies. Another set of immune molecules, the T-cell factors regulating antibody production², have not previously been extensively characterised, mainly because of the lack of homogenous and reproducible sources. The use of T-cell hybrids³⁻⁵ which would produce large amounts of a given factor would therefore be very useful for the characterisation of these molecules. Among the T-cell factors regulating antibody production, Gisler and W.H.F.⁶ have described an immunoglobulin binding factor (IBF) which binds to IgG, not to IgM and suppresses antibody production to T-dependent and T-independent antigens nonspecifically *in vitro*. IBF is the soluble form of the T-cell Fc γ receptor⁷ which is expressed on non antigen-specific suppressor T cells^{8,9}. As the Fc γ receptor is a marker of a set of suppressor T cells, one would expect that selection of T-cell hybrids for the expression of this receptor may lead to selection of T-cell lines producing permanently non antigen-specific suppressor factor. We report here the establishment of T-cell hybrids which express Fc γ receptors and produce suppressor IBF.

T1 hybrid cell line and clones were obtained by fusion of BW 5147 T lymphoma cells (hypoxanthine, aminopterin and thymidine (HAT) sensitive, H-2^k Thy-1.1 positive, Fc γ receptor negative) with spleen cells from C57BL/6 (H-2^b) mice primed with sheep red blood cells (SRBC). The T2C2 and T2D4 cell lines were obtained by fusion of the BW 5147 T lymphoma cells with B10.BR alloantigen-activated T cells (H-2^k, Thy-1.2 positive and 40% Fc γ receptor positive). Cells were fused using polyethylene glycol (PEG) as a fusion agent (Table 1). After distribution in microplates and two weeks of selection in HAT medium, hybrid populations were grown in RPMI 1640 medium (Eurobio) containing 10% fetal calf serum (FCS) (Eurobio) (RPMI FCS). The hybrids were selected for the presence of membrane Fc γ receptors by a rosette technique and tested for the expression of H-2, Thy-1 and Lyt alloantigens by microcytotoxicity assays. Rosette determinations were carried out at 4°C using sheep erythrocytes (E) sensitised with IgG (EAIGG) or IgM (EAIGM) rabbit anti-Forssman antibodies⁷. The clones

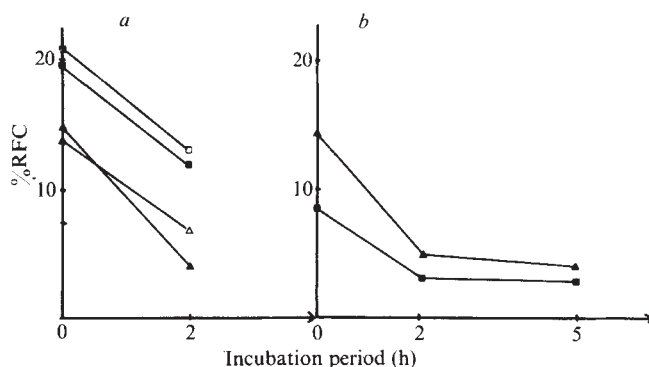


Fig. 1 Release of Fc receptors from T-cell hybrids. Hybrid cells were incubated from 2-5 h at 37°C at a concentration of 2×10^6 cells per ml and tested for their capacity to form rosettes with EAIGG. Rosette determinations were performed at 4°C by mixing 5×10^5 hybrid cells with 10^7 IgG-sensitised erythrocytes in 0.5 ml BSS. After centrifugation at 1,000 r.p.m. for 10 min at 4°C, and gentle resuspension, rosettes were counted. a, $\square$, T1C1E5; $\blacksquare$, T1; $\triangle$, T1C1E5; $\blacktriangle$, T2D4. RFC, Rosette-forming capacity.

Table 1 Karyotic analysis and cell surface markers of T-cell hybrid lines

Hybrid cell lines*	Parental populations†		Average no. of chromosomes‡	Cell surface markers§
T1	AKR T lymphoma BW5147 (H-2 ^k , Thy-1.1)	× Spleen cells from C57BL/6 mice immunised with SRBC (H-2 ^b , Thy-1.2, Lyt-1.2, Lyt-3.2)	53	H-2 ^b , H-2 ^k , Thy-1.2, Thy-1.1, Lyt-3.2
T2C2 and T2D4	AKR T lymphoma BW5147	× B10.BR <i>in vivo</i> alloantigen-activated T cells (H-2 ^k , Thy-1.2)	51	H-2 ^k , Thy-1.1, Thy-1.2

* Fusion was performed by adding 1 ml of 50% PEG (w/v) 1500 in serum-free RPMI to a pellet of a mixture of 10^8 spleen cells and 10^7 BW5147 cells³. After 1 min at 37°C, the suspension was gradually diluted to 10 ml (in 5 min) with serum-free RPMI 1640. After centrifugation RPMI FCS was added and cell suspension was distributed in 0.2 ml microplates (Falcon no. 3040) at a concentration of 2×10^6 cells per well and grown in selective HAT medium in a CO₂ incubator (5% CO₂) in air. Two weeks later, selective medium was removed and replaced by RPMI FCS.

† Spleen cells of C57BL/6 mice were collected 5 days after immunisation by intraperitoneal injection of 10^8 SRBC. Alloantigen-activated B10.BR T cells were taken from spleens of lethally irradiated (850 rad) BALB/c mice reconstituted 5 d before by intravenous injection of 10^8 B10.BR thymocytes.

‡ The average number of chromosomes of BW5147 was 43. In each experiment 15 to 21 mitoses were observed.

§ The hybrid cell lines were negative for cell surface Ig as revealed by immunofluorescence. The detection of other cell surface markers was performed by a microcytotoxicity technique using guinea pig serum as a source of complement. 100% cells of T1 hybridoma were killed by C3H anti-C57BL/6 (anti-H-2^b) antiserum up to 1/80 dilution, by C57BL/6 anti-C3H (anti-H-2^k) antiserum up to 1/160 dilution, by AKR anti-C3H (anti-Thy-1.2) antiserum up to 1/40 dilution, by C3H anti-AKR (anti-Thy-1.1) antiserum up to 1/20 dilution and by C58 anti-C58.CE Lyt-3.2 (anti-Lyt-3.2) antiserum up to 1/20 dilution. It was resistant to lysis by C3H anti-C3H.CE Lyt-1.2 (anti-Lyt-1.2) antiserum. 100% cells of T2C2 and T2D4 were killed by C57 anti-C3H (anti-H-2^k) antiserum up to 1/160 dilution, by C3H anti-AKR (anti-Thy-1.1) antiserum up to 1/20 dilution, and by AKR anti-C3H (anti-Thy-1.2) antiserum up to 1/20 dilution.

T1C1C5, T1C1E4 and T1C1E9 from the Fc γ receptor-positive T1 hybrid were obtained by distribution of individual cells in microplates and were grown in RPMI FCS.

In a first series of experiments we established that T1 and T2 are T-cell hybrids. As shown in Table 1, their number of chromosomes (50–54) is higher than the number of chromosomes of the BW 5147 lymphoma (41–45), and they express H-2 and Thy-1 alloantigens of both parental strains and no cell surface immunoglobulins. In addition, T1 hybrid cells were found to be positive for Lyt-3.2 antigen which is known to be a marker of cytotoxic and suppressor T cells and to be negative for Lyt-1.2 antigen. With regard to the presence of Fc γ receptors, T1 hybrid, its clone derivatives (T1C1C5, T1C1E4, T1C1E9) and the several T2 hybrid populations (T2C2, T2D4 and five others) showed a permanent (over 12 months) capacity to form rosettes with EAIGG, while the parental BW cell line was negative. These rosettes detect specifically the Fc γ receptors as: (1) 100% are inhibited by aggregated rabbit and mouse IgG and not by aggregated F(ab')₂ fragments of rabbit IgG or by mouse IgM; and (2) no rosettes were found with E or EAIGM (data not shown).

In previous studies we have shown that, when activated T cells are incubated at 37°C, the Fc γ receptors disappear from the cell surface and suppressor IBF appears in the supernatant¹⁰. We therefore tested whether hybridomas would exhibit a similar phenomenon. When taken from the cultures and washed, 6–35% of cells of the different hybrid cell lines could form EAIGG rosettes depending on the phase of the culture. As shown in Fig. 1, after incubation of the hybrid cells for 2–5 h at 37°C at a concentration of 2×10^6 cells per ml in serum-free BSS, the number of EAIGG rosette-forming cells (EARFC) was much decreased. This was not due to death of Fc γ receptor-positive cells as viability remains constant throughout the incubation period, but rather suggests that the hybrid cell lines, like the activated T cells⁷, may release Fc γ receptors into the medium.

To determine if suppressor IBF is produced by the hybrid cells in these conditions, supernatants of 2×10^6 cells incubated for 2 h at 37°C in serum-free BSS were tested for the presence of a suppressor factor which could be retained on IgG immunoadsorbents. Table 2 shows representative experiments. In most cases, crude supernatants of the hybrid cell lines (T1C1C5, T1C1E4, T1C1E9, T2C2 and T2D4) suppressed *in vitro* antibody production to SRBC and this suppressive activity was recovered in the acid eluates of IgG immunoadsorbents but not in the effluents. In one case (T1), even if no suppressive activity could be found in the crude supernatant, the acid eluate and not the effluent strongly inhibited the PFC response. As a control, neither the crude supernatants nor their acid eluates of BW 5147 parental cell line exerted suppressive activity. As the acid eluates contain only molecules which bind to IgG—IBF by definition—and the effluents are specifically devoid of these molecules, it is possible to state that the Fc γ receptor-positive T-cell hybrids produce suppressor IBF.

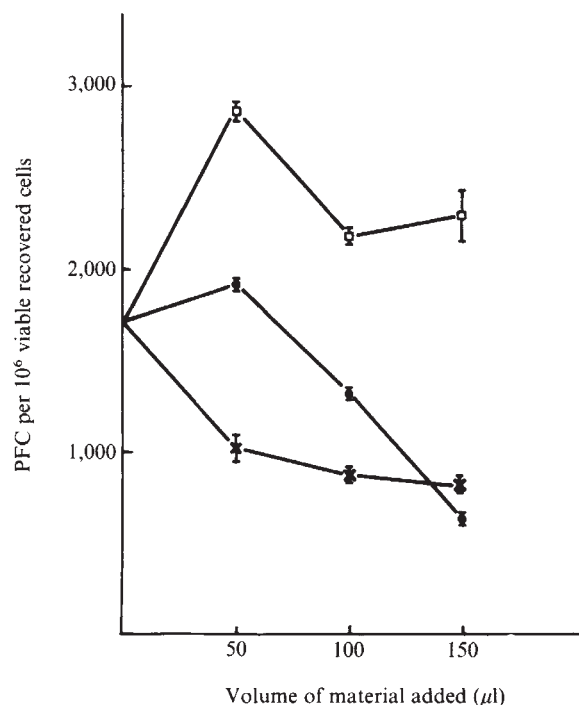


Fig. 2 Titration of suppressive IBF produced by T1 hybrid cells. Graded doses of crude supernatants (●), effluents (□) and acid eluates (×) from IgG immunoadsorbents were added 48 h after antigen to spleen cell cultures of C57BL/6 mice stimulated by SRBC, using a technique in tubes⁶. Direct PFC were measured at day 5 by the local haemolysis technique in liquid medium¹¹. Each culture was made in duplicate and results are expressed as PFC per 10^6 viable recovered cells.

The amount of suppressor factor produced by the hybrids is similar to the amount of suppressive IBF produced by activated T cells, as when supernatants and acid eluates from activated T cells or hybrid cells were prepared in the same way and tested at the same dose, no significant differences could be detected (Table 2). To titrate the suppressor factor produced by hybrid cells more precisely, graded doses of supernatants, effluents and acid eluates from IgG immunoadsorbents were added to spleen cells stimulated with SRBC. Figure 2 shows a representative experiment using T1 hybrid. A dose-dependent inhibition of antibody production was obtained with supernatant and acid eluate, while no suppression was observed with the effluents. Similarly to T-cell-produced IBF⁶, 100% inhibition was never found, but 60–70% were obtained with small doses of acid eluate and high doses of crude supernatant, indicating enrichment in suppressive activity. The binding of suppressor factor

Table 2 Production of suppressive IBF by T1, its clones derivatives and T2 hybrid cell lines

Material added to cultures of spleen cells stimulated by SRBC*	Cell lines							
	T1		T1C1C5		T1C1E4		T1C1E9	
	PFC/10 ⁶ cells	% In	PFC/10 ⁶ cells	% In	PFC/10 ⁶ cells	% In	PFC/10 ⁶ cells	% In
None	18,975 ± 1380	—	23,064 ± 1726	—	23,064 ± 1726	—	23,064 ± 1726	—
Supernatant†	13,129 ± 96	31	14,950 ± 1150	35	9,200 ± 767	56	9,009 ± 384	61
Effluent from IgG-coated Sepharose beads‡	21,850 ± 383	0	ND	—	ND	—	ND	—
Acid eluate from IgG-coated Sepharose beads‡	8,740 ± 575	54	10,022 ± 1315	57	7,962 ± 885	67	8,913 ± 239	61

Material added to cultures of spleen cells stimulated by SRBC*	Cell lines							
	T2C2		T2D4		BW		Activated cells	
	PFC/10 ⁶ cells	% In	PFC/10 ⁶ cells	% In	PFC/10 ⁶ cells	% In	PFC/10 ⁶ cells	% In
None	2,597 ± 245	—	2,597 ± 245	—	6,210 ± 190	—	26,105 ± 6731	—
Supernatant†	623 ± 144	76	1,186 ± 36	54	8,881 ± 64	0	11,131 ± 945	57
Effluent from IgG-coated Sepharose beads‡	2,714 ± 322	0	2,185 ± 460	16	ND	—	ND	—
Acid eluate from IgG-coated Sepharose beads‡	1,093 ± 173	58	1,416 ± 266	45	5,842 ± 129	6	1,0570 ± 398	60

* 8×10^6 spleen cells from B6D2F1 mice were cultured in RPMI 1640 supplemented with 10% FCS (Rehatuin-Reheis), 1% horse serum and antibiotics (penicillin, streptomycin) in Falcon plastic tubes for 5 d in a CO₂ incubator (5% CO₂ in air)⁶. SRBC (3×10^6 cells) were added at day 0, 100 μ l of materials were added 48 h later and direct PFC were enumerated at day 5 by the liquid local haemolysis technique¹¹. PFC are expressed per 10⁶ viable recovered cells.

† Hybrid cells or activated T cells were washed at 4 °C in BSS and incubated at a concentration of 2×10^6 cells ml⁻¹ for 2 h at 37 °C in serum-free BSS. Cell-free supernatants were taken after centrifugation at 200g for 10 min.

‡ Cyanogen bromide-activated Sepharose beads (Pharmacia) were coupled with 5 mg ml⁻¹ of rabbit IgG (Miles, Eurobio) in sodium bicarbonate buffer, 0.1 M pH 8 (ref. 12). For immunoadsorption, a batch technique was used: 2 volumes of supernatants were mixed with 1 volume of Sepharose beads for 2 h at 22 °C, and the effluents were recovered after centrifugation. After washings in phosphate buffer, 0.02 M pH 7, the beads were incubated in 0.2 M glycine HCl pH 2.8 buffer and the eluates were recovered by centrifugation and neutralised to pH 7. Effluents and eluates were concentrated to half starting volume, dialysed against BSS, and sterilised on 0.22 μ m Millipore filter.

|| Alloantigen-activated T cells were prepared as described in legend to Table 1.

ND, Not determined. % In, % inhibition. Thirty per cent inhibition was considered significant.

specifically to IgG and its non-antigen specificity is shown in Table 3. First, in experiment 1, crude supernatant of the T1 hybrid cell line inhibits 21% of the plaque-forming cell (PFC) response, and all the suppressive activity is present in the acid eluate of an IgG immunoabsorbent and not in the effluent. By contrast, when the same supernatant is applied to an IgM immunoabsorbent, suppressive activity is recovered in the effluent and not in the eluate, indicating that the suppressor factor binds specifically to IgG. As T1 hybrid was prepared with spleen cells sensitised with SRBC, it was necessary to rule out the presence of an antigen-specific suppressor factor in the eluates. Experiment 2 in Table 3 shows that the suppressive activity found in acid eluates from IgG immunoabsorbents could not be adsorbed on SRBC, demonstrating that IBF produced by T-cell hybrids as well as IBF produced by activated T cells behave as a non-antigen-specific suppressor factor.

T-cell hybrids have already been obtained by several laboratories³⁻⁵ but in most cases, except for one hybrid producing an antigen-specific suppressor factor¹³, immunological functions of parental spleen cells were lost after fusion. A recent report by Taniguchi and Miller¹⁴ describes several T-cell hybridomas obtained by using parental spleen cells enriched for I-J sub-region markers and containing specific and nonspecific suppressor factors. However, suppressive activity was lost after 3 months of culture. The present work shows that selection of T-cell hybrids for the presence of Fc γ receptors leads to the establishment of lines which produce permanently (over 12 months) non-antigen-specific suppressor factor. Also, some T lymphomas have been shown to permanently produce immunoregulatory molecules¹⁵ and in particular, Fc receptors^{16,17}. However, first, these molecules are of tumour and not T-cell origin and may not be identical to physiological products, and second, hybridisation of a given lymphoma with selected T-cell subpopulations may lead to T-cell lines producing various

factors which could be compared on a biochemical basis and whose eventual differences could not be due to different origin of the tumour cells. These T-cell lines may be very useful for the characterisation of factors and for *in vivo* studies.

Table 3 Production of an IgG binding, non-antigen-specific, suppressor IBF by T1 hybrid cell line

Material added to cultures of spleen cells stimulated by SRBC*	PFC per 10 cells	% Inhibition
Expt 1		
None	6,210 ± 190	—
Supernatant	4,910 ± 152	21
Effluent from IgG-coated Sepharose beads	5,485 ± 531	12
Acid eluate from IgG-coated Sepharose beads	2,492 ± 192	60
Effluent from IgM-coated Sepharose beads*	3,368 ± 411	46
Acid eluate from IgM-coated Sepharose beads*	5,134 ± 1,355	17
Expt 2		
None	3,283 ± 293	—
Acid eluate from IgG-coated Sepharose beads	2,058 ± 80	37
Acid eluate from IgG-coated Sepharose beads adsorbed with SRBC‡	2,052 ± 166	37

* See Table 2 legend for details.

‡ One volume of packed SRBC were incubated with 3 volumes of eluate for 1 h at 4 °C and centrifuged at 2,000 r.p.m. before use.

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Novel α_2 -adrenoreceptors primarily responsible for inducing human platelet aggregation

BLOOD PLATELETS isolated from humans, in contrast to those from other mammalian species, aggregate on exposure to adrenaline or noradrenaline¹. Studies using α antagonists have shown that this effect results from occupancy of an α adrenoreceptor^{2,3}. However, Jakobs has reported that selective α agonists such as clonidine and phenylephrine bind with high affinity to the platelet α adrenoreceptor^{4,5}, but fail either to induce an aggregation response⁶ or to inhibit platelet adenylate cyclase⁶, in contrast to the effects of natural agonists such as adrenaline^{1,7}. Instead, such α agonists seem to act as antagonists at the platelet adrenoreceptor⁶. α Adrenoreceptors in other tissues have been classified as α_1 (postsynaptic) or α_2 (presynaptic) on the basis of their affinities for various selective α agonists and antagonists⁸. Jakobs⁶ has, however, interpreted his data on the human platelet adrenoreceptor as indicating the existence of a third category of α adrenoreceptor at which only the natural catecholamines are agonists. We report here that we have confirmed the observation that clonidine and phenylephrine are ineffective in inducing aggregation of human platelets and act as inhibitors of the response to adrenaline. However, further examination has revealed that the effects of these selective α agonists differ in certain important respects from those of selective α antagonists, thus requiring substantial modification of the thesis advanced by Jakobs⁶.

Figure 1 shows the effect of clonidine and of yohimbine, a selective α_2 antagonist^{9,10}, on the aggregation response of human platelets to ADP, vasopressin and serotonin. Addition of clonidine 15 s before each of these agonists causes marked stimulation of both phases of the response. Stimulation of the response is also observed if clonidine is added before other

agonists, such as collagen, thrombin or arachidonate. From the detailed dose-response curve (Fig. 1a) the concentration of clonidine which gives half-maximal stimulation of aggregation induced by ADP is 0.6 μ M, in agreement with the concentration of this selective α agonist which gives half-maximal inhibition of the primary response to adrenaline (0.6 μ M). In contrast, yohimbine, which is also an effective inhibitor of aggregation induced by adrenaline (Fig. 2a), has little effect on aggregation induced by most other agonists (compare Fig. 1b and c) at concentrations which completely inhibit the response to adrenaline, and notably fails to stimulate these responses. The only exception is the response to serotonin where the extent of inhibition by yohimbine is rather more marked (Fig. 1d). However, yohimbine is an effective inhibitor of the stimulation by clonidine of the response to other agonists (such as ADP, vasopressin, serotonin) (Fig. 1b-d). The concentration of yohimbine required for half-maximal inhibition of stimulation by clonidine of the response to ADP (2.5 μ M) is, however, significantly greater than that which causes half-maximal inhibition of the primary phase of the response to adrenaline (0.5 μ M).

Similar studies have been carried out using phenylephrine and the selective α_1 antagonist, indoramin^{12,13}. Phenylephrine added 15 s before platelet agonists other than adrenaline also

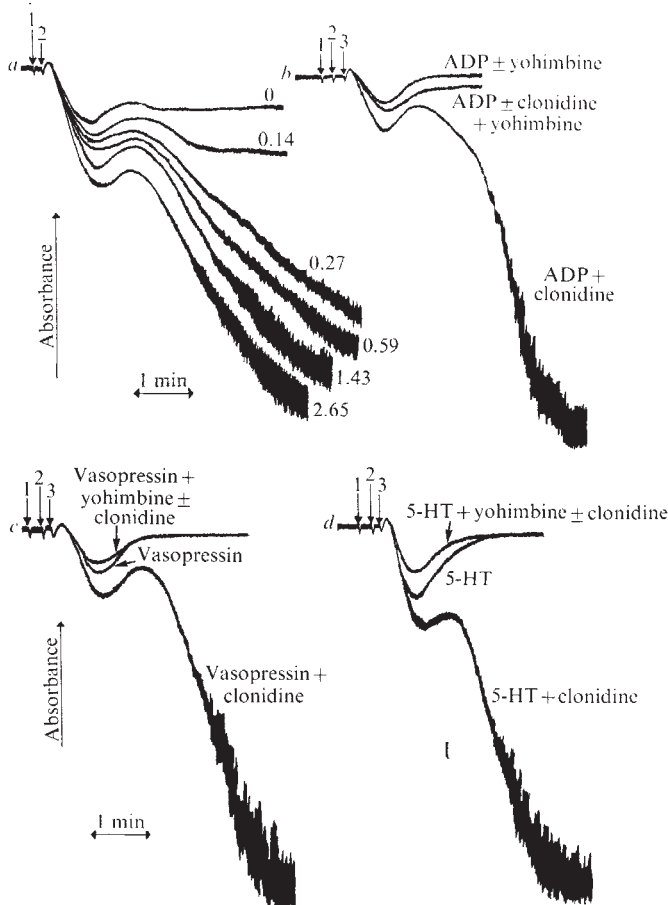


Fig. 1 Stimulation by clonidine of the response of human platelets to ADP, vasopressin and serotonin (5-HT) and the inhibition of this effect by yohimbine. Platelet-rich plasma was obtained from drug-free donors using 0.1 volumes of acid-citrate dextrose (0.1 M citrate) as anticoagulant, and platelet aggregation was monitored using a Payton dual-channel aggregometer as described previously¹¹. Additions were as follows: a, at 1, clonidine at the concentrations (μ M) indicated by the numbers on the traces; at 2, 1.25 μ M ADP; b, at 1 when indicated, 5 μ M yohimbine, at 2 when indicated, 5.7 μ M clonidine, at 3, 0.75 μ M ADP; c, at 1 when indicated, 10 μ M yohimbine, at 2 when indicated, 5.7 μ M clonidine, at 3, 0.046 nM vasopressin; d, at 1 when indicated 10 μ M yohimbine, at 2 when indicated, 5.7 μ M clonidine, at 3, 1 μ M serotonin. Absorbance is in arbitrary units.

causes a stimulation of the response to these agonists which is similar in magnitude to that observed in the presence of clonidine (Fig. 1). This is shown in Fig. 3 for ADP, thrombin and arachidonate. The concentration of phenylephrine which gives half-maximal stimulation of the response to ADP (30 μM) (Fig. 3a) is in reasonable agreement with that which gives half-maximal inhibition of the response to adrenaline (25 μM), although we did not obtain complete inhibition of the adrenaline response by phenylephrine at concentrations where the effect is specific. In contrast, indoramin fails to stimulate the response of human platelets to ADP or thrombin over a range of concentrations which causes inhibition of the response to adrenaline (Fig. 3b, c). The concentration of indoramin required to cause 50% maximal inhibition of the adrenaline response is 42 μM (Fig. 2b). Although 150 μM indoramin causes complete inhibition of the response to adrenaline the effect may represent a nonspecific action, as this concentration also causes complete inhibition of the response to ADP. Specific inhibition of the adrenaline response is only observed at indoramin concentrations $<50 \mu\text{M}$. Both yohimbine and indoramin selectively inhibit phenylephrine stimulation of the response to ADP (Fig. 3b). The concentrations of these antagonists required to give half-maximal inhibition of the stimulated response are 27 and 2 μM , respectively. Indoramin also selectively blocks phenylephrine-induced stimulation of the response to thrombin (Fig. 3c).

The stimulation of the response of human platelets to agonists such as ADP, vasopressin, serotonin and thrombin which results from the addition of the specific α agonists (Figs 1, 3) resembles that reported previously for rat platelets in which phenylephrine was found to enhance the response to ADP¹⁴. The effect in human platelets is quantitatively similar to that observed when a non-aggregating concentration of adrenaline is added before any one of these agonists^{2,15}. As selective α antagonists fail to stimulate the response of human platelets to agonists other than adrenaline but act as inhibitors with respect to adrenaline itself, selective α agonists such as clonidine and phenylephrine seem to interact as partial agonists at the platelet adrenoceptor rather than as antagonists as suggested by Jakobs⁶. This situation, although unusual, is not unique, as other systems have been described in which clonidine at least, seems to act as a partial agonist¹⁶.

Our data also provide insight into the nature of the adrenoceptors on human platelets that are responsible for initiation of the aggregation response. As yohimbine is two orders of magnitude more potent than indoramin as an antagonist of aggregation induced by adrenaline (Fig. 2), this latter response seems to be mediated by α_2 adrenoceptors. A similar conclusion is applicable to the action of clonidine as a partial agonist in

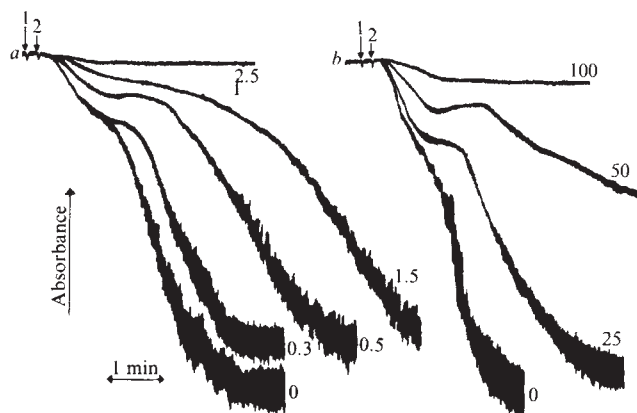


Fig. 2 Inhibition by yohimbine and indoramin of the response of human platelets to adrenaline. Platelet aggregation was monitored as described for Fig. 1. Additions were as follows: a, at 1, yohimbine at the concentrations (μM) indicated by the numbers on the traces; at 2, 5 μM adrenaline; b, at 1, indoramin at the concentrations (μM) indicated by the numbers on the traces; at 2, 5 μM adrenaline.

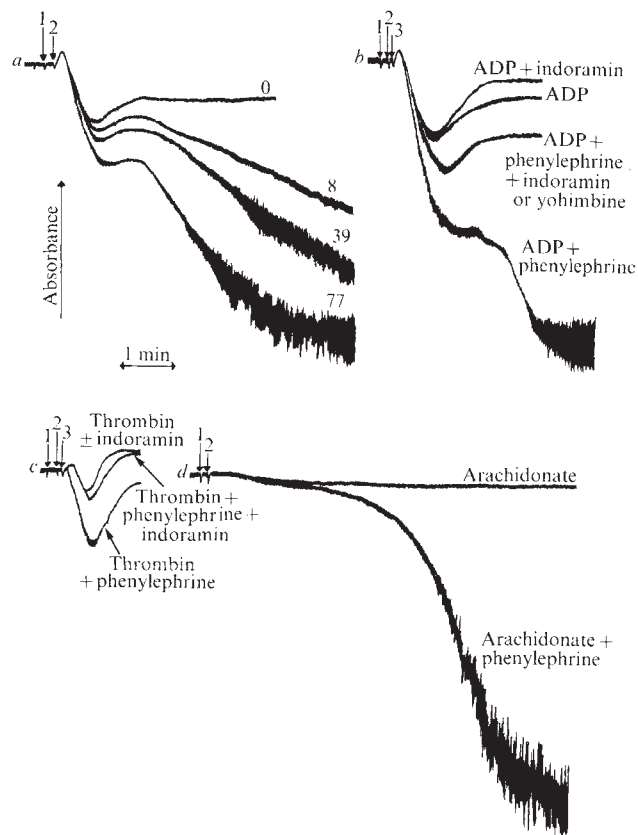


Fig. 3 Stimulation by phenylephrine of the response of human platelets to ADP, thrombin and arachidonate and the inhibition of this effect by yohimbine and indoramin. Platelet aggregation was monitored as described for Fig. 1. Additions were as follows: a, at 1, phenylephrine at the concentrations (μM) indicated by the numbers on the traces; at 2, 1.25 μM ADP; b, at 1 when indicated, 10 μM indoramin or 40 μM yohimbine, at 2 when indicated, 77 μM phenylephrine and at 3, 1.5 μM ADP; c, at 1 when indicated, 5 μM indoramin, at 2 when indicated, 77 μM phenylephrine; at 3, 0.28 units thrombin; d, at 1 when indicated, 38.5 μM phenylephrine; at 2, 0.4 μM arachidonate.

stimulating the response to agonists other than adrenaline, although in this case, blockade by yohimbine occurs over a range of concentration significantly higher than that required for inhibition of the response to adrenaline (Figs 1, 2). In contrast, indoramin at concentrations $<50 \mu\text{M}$ has little effect as an inhibitor of the clonidine-stimulated responses, as would be expected from the relative efficiency of the two antagonists on the response to adrenaline (Fig. 2). However, the pattern of inhibition by indoramin and yohimbine is exactly reversed for phenylephrine stimulation of the response to agonists other than adrenaline. In this case, complete blockade of the stimulated response by indoramin occurs at a concentration (5–10 μM) which has little, if any, effect on aggregation induced by adrenaline (Figs 2b, 3b, c). Blockade by yohimbine of the phenylephrine stimulation is, in contrast, less effective than that caused by indoramin and occurs only at concentrations of yohimbine well in excess of those required for inhibition of the responses to adrenaline or clonidine. This pattern of inhibition suggests that, in contrast to adrenaline or clonidine, phenylephrine is exerting its effects through α_1 adrenoceptors which are, however, less effective in inducing aggregation and make only a minor contribution to the response to the natural agonist.

Both our data and those of Jakobs⁶ seem, therefore, to be satisfied by a postulate which suggests that human platelets carry both α_1 and α_2 adrenoceptors at which phenylephrine and clonidine, respectively, act as partial agonists. The α_2 adrenoceptor is identified as being primarily responsible for mediating the response to the natural agonist. This conclusion seems to

agree with the nature of the response of human platelets to adrenaline that involves secretion as well as aggregation^{2,8}. The proposal by Jakobs⁶ that the platelet carries a unique species of adrenoreceptor does not seem warranted by the data now available.

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Amines inhibit the clustering of α_2 -macroglobulin and EGF on the fibroblast cell surface

α_2 -MACROGLOBULIN (α_2 M), insulin and epidermal growth factor (EGF) have a common mechanism for internalisation¹. We have proposed a model for this mechanism¹⁻³ in which the ligands bind to diffusely distributed receptors followed by the collection of the mobile ligand-receptor complexes over coated pits. These coated pits pinch off from the plasma membrane to form endocytic vesicles. The diffuse initial distribution of the α_2 M receptors has been shown by binding α_2 M to fixed cells and observing the location of α_2 M by an electron microscopic immunocytochemical method². The mobility of the ligand-receptor complexes has been demonstrated using fluorescence photobleaching recovery⁴, and the clustering of such complexes has been demonstrated by fluorescence microscopy^{1,3,5}. Electron microscopic immunocytochemical localisation has been used to show that the clustering occurs over coated pits which pinch off to form endocytic vesicles². The mechanism proposed for the endocytosis of α_2 M, insulin and EGF is similar to that described for the internalisation of low density lipoprotein (LDL), except that unoccupied LDL receptors may cluster in coated pits before binding LDL⁶. It seems likely that many molecules, including other polypeptide hormones and the lysosomal hydrolases⁷ which bind to specific receptors on the cell surface, are internalised by a similar mechanism. To determine the role of clustering and internalisation in hormone action it would be useful to have an agent that inhibits these processes. It has been found that the internalisation of lysosomal enzymes is noncompetitively inhibited by some amines and ammonia (E. Neufeld, personal communication). It has also been reported that ammonia prevents the internalisation of diphtheria toxin by HeLa cells⁸. In view of these effects, we examined the possibility that amines and ammonia would block the clustering of α_2 M and EGF, and we report here that these agents are effective in blocking clustering and internalisation.

When rhodamine-labelled α_2 -macroglobulin (R- α_2 M) is added to the culture medium of Swiss 3T3-4 cells, the

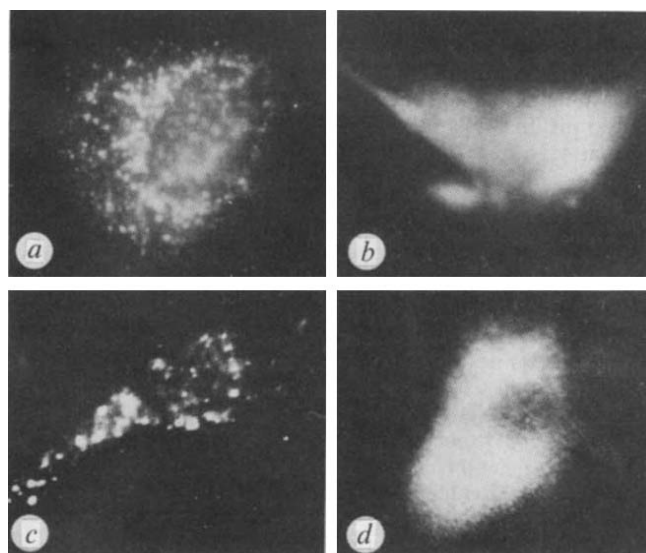


Fig. 1 Inhibition of α_2 M clustering. *a*, Swiss 3T3-4 cells were incubated for 20 min at 37 °C in Dulbecco-Vogt's modified Eagle's medium containing 30 μ g ml⁻¹ R- α_2 M. *b*, Cells were incubated with 10 mM methylamine hydrochloride for 20 min at 37 °C and 30 μ g ml⁻¹ R- α_2 M was then added for a further 20 min incubation at 37 °C. In *c*, 30 mM methylamine was present before and during the 20 min incubation with R- α_2 M. The cells were then rinsed with fresh medium and incubated for 20 min at 37 °C. This resulted in a punctate fluorescence pattern similar to that in *a*. When methylamine was present for the final incubation (not shown), the fluorescence remained diffuse as in *b*. *d*, Cells were incubated in medium without Ca²⁺, containing 1 mM Mg²⁺ and 1 mM EGTA, for 5 min at 37 °C. 30 μ g ml⁻¹ R- α_2 M was then added, and the cells were incubated for 30 min at 37 °C. After the indicated incubations, the cells were rinsed and fixed with 2% formaldehyde in Dulbecco's phosphate-buffered saline for 1 min at 23 °C. The fluorescence was recorded on videotape using a silicon intensifier target television camera⁵, and photographs were taken from the video monitor. Magnification, $\times 678$.

fluorescent emission from the R- α_2 M is observed in a punctate distribution on the cells⁵ (Fig. 1*a*) as a result of clustering and endocytosis^{1,2}. In contrast to the punctate fluorescence seen in Fig. 1*a*, R- α_2 M added after a brief incubation with methylamine forms a diffuse pattern of fluorescence on the cells (Fig. 1*b*). No inhibition of clustering was noted at amine concentrations below 0.1 mM, and the inhibition of clustering is complete at 10–30 mM methylamine hydrochloride.

Ammonium acetate and the hydrochloride salts of methylamine, ethylamine, propylamine and *n*-butylamine were all effective in blocking clustering, and all showed a similar concentration dependence. *t*-Butylamine was slightly less effective than these amines in blocking clustering. Chloroquine (0.1 mM), lidocaine (1 mM), Tris-HCl (30 mM), *N*-acetyl-L-lysine-*N'*-methylamide (10 mM) and 1, 4-diaminobutane-2HCl (putrescine) (10 mM) did not block clustering. It is apparently necessary for the amines to be inside the cells for the clustering to be inhibited, as simultaneous addition of methylamine and R- α_2 M resulted in extensive clustering. The failure of putrescine to block clustering is probably due to its low rate of uptake⁹, compared to aliphatic monoamines, which diffuse through the membrane as the uncharged species.

The fluorescence intensity of cells incubated with R- α_2 M in the presence or absence of methylamine was approximately the same, indicating that the binding of α_2 M by surface receptors is not blocked by the amine. Furthermore, R- α_2 M which was bound diffusely to the cells in the presence of methylamine appeared in a punctate distribution 20 min after removing the amine from the medium (Fig. 1*c*). Fluorescence photobleaching recovery measurements^{4,10} (F.R.M., unpublished) demonstrated that amines did not reduce the mobility of α_2 M-receptor complexes.

The inhibition of clustering by methylamine was confirmed using immunocytochemical localisation. Figure 2a demonstrates the diffuse labelling of the plasma membrane. There was an occasional slight concentration of label in these conditions in a coated pit, but this degree of clustering is considerably less than that seen in the absence of methylamine (Fig. 2b). The morphology of the cells was not changed in any obvious way by methylamine treatment.

Removal of Ca^{2+} from the medium was also found to inhibit the clustering of $\text{R-}\alpha_2\text{M}$ (Fig. 1d). The $\text{R-}\alpha_2\text{M}$ bound to the cells in the absence of extracellular Ca^{2+} did not form clusters when fresh medium containing Ca^{2+} (without $\text{R-}\alpha_2\text{M}$) was added to the cells. However, the cells were still able to form clusters after EGTA treatment; $\text{R-}\alpha_2\text{M}$ added with fresh Ca^{2+} -containing medium formed clusters normally. EGTA itself was not responsible for the inhibition of clustering. When excess (4 mM) Ca^{2+} was added to medium containing 1 mM EGTA, $\text{R-}\alpha_2\text{M}$ formed clusters on the cells. Magnesium was not able to substitute for calcium. Evidently, calcium must be present as the $\alpha_2\text{M}$ binds to its cell surface receptor, as addition of calcium after removal of the $\text{R-}\alpha_2\text{M}$ from the medium did not result in clustering.

EGF has previously been shown to form clusters which coincide with the clusters formed by $\alpha_2\text{M}^{1,3}$. Figure 3 shows that 30 mM methylamine blocks the clustering of a rhodamine-labelled derivative of EGF. As with $\alpha_2\text{M}$, binding to cell surface receptors was not inhibited by methylamine.

It has been suggested that ammonia, chloroquine and lidocaine might block the degradation of EGF by the same mechanism, perhaps by inhibition of proteolysis in lysosomes¹¹. Our results indicate that the proximal effect of ammonia (but not chloroquine or lidocaine) in blocking EGF degradation is the inhibition of clustering. Ammonia also blocks the degradation of human choriongonadotropin (HCG) by murine Leydig tumour cells, without affecting HCG-induced steroidogenesis¹². We

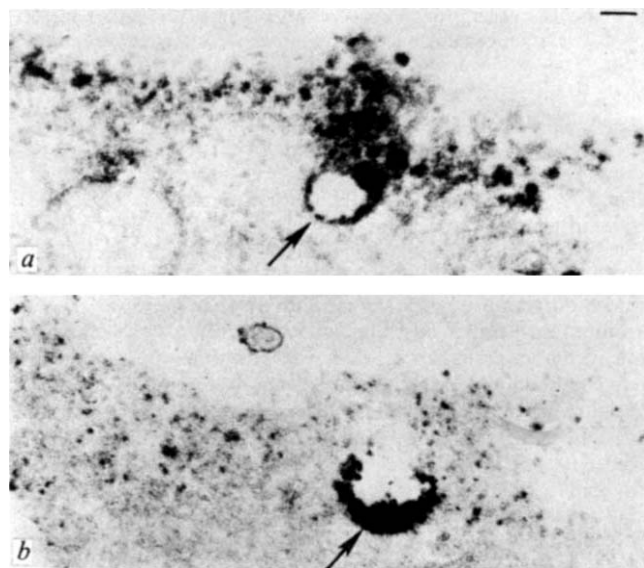


Fig. 2 Localisation of surface-bound $\alpha_2\text{M}$. Cells were pretreated with 30 mM methylamine (a) for 20 min at 37 °C followed by incubation with 250 $\mu\text{g ml}^{-1}$ $\alpha_2\text{M}$ for 5 min. They were then rinsed and fixed with 2% glutaraldehyde, and the surface-bound $\alpha_2\text{M}$ was detected using affinity-purified antibody to $\alpha_2\text{M}$ and peroxidase-conjugated anti-IgG (Cappel). Cells were incubated with diaminobenzidine substrate, post-fixed in 1.5% OsO_4 , dehydrated in ethanol and embedded in Epon 812 as previously described². Tangential thin sections were viewed without staining at 50 kV with a Hitachi HU-12A electron microscope. In a the diffuse labelling of the surface in 30 mM methylamine is shown, and a coated pit (arrows) shows little or no concentration of label above the rest of the surface. This is in marked contrast to the massive concentration of label in the coated pits in the absence of methylamine (b). Magnification, $\times 50,400$; bar, 0.1 μm .

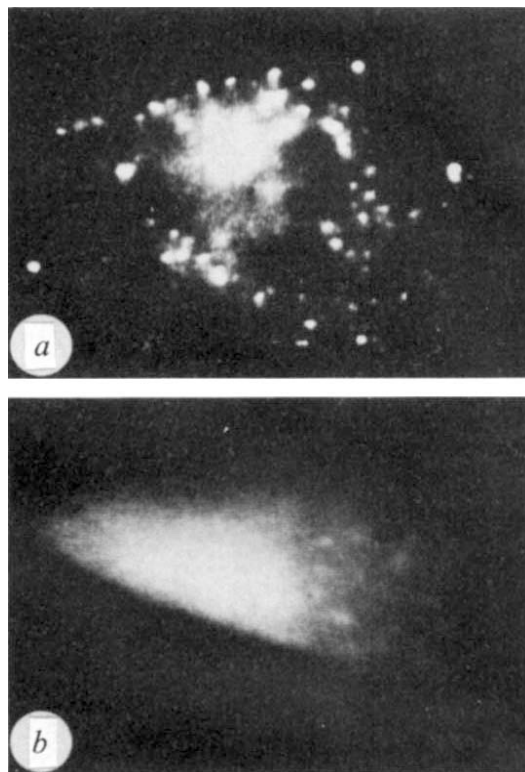


Fig. 3 Methylamine inhibition of EGF clustering. Cells were incubated in medium containing 1% fetal calf serum without (a) or with (b) 30 mM methylamine for 20 min at 37 °C. 50 ng ml^{-1} of rhodamine-labelled EGF was then added to the cells, and the cells were incubated for 20 min at 37 °C. They were then rinsed and fixed, and fluorescence was recorded as in Fig. 1. The rhodamine-labelled EGF consisted of rhodamine-labelled α -lactalbumin coupled to the α -amino group of EGF. The binding affinity of this derivative was shown to be the same as that of native EGF¹⁶. Magnification, $\times 1,130$.

speculate that the internalisation of this hormone is blocked by ammonia.

The mechanism for blocking clustering is not clear. One possible mechanism of action for the amines would involve an increase in the intracellular pH resulting from diffusion of uncharged amines across the plasma membrane followed by protonation inside the cell. It has been shown that chloroquine, methylamine and ammonia raise the lysosomal pH in this way¹³. The increase in pH could then, for example, interfere with a protein-protein interaction necessary for clustering.

An alternative is that the enzyme transglutaminase is involved in the formation of clusters of receptor-ligand complexes over coated pits. This enzyme catalyses the formation of ϵ -(γ -glutamyl) lysine crosslinks between proteins¹⁴. It requires calcium for activity and is inhibited by ammonia and aliphatic amines, both of which are properties shared by the mechanism for the clustering of receptor-ligand complexes. ϵ -(γ -glutamyl) lysine crosslinks have been found in high concentrations in the plasma membrane proteins of mouse L cells¹⁵. According to this hypothesis, the binding of a ligand to its receptor would activate endogenous transglutaminase (through Ca^{2+} binding to the enzyme), which would then crosslink the receptor (or an associated protein) into the coated pit.

The inhibition of clustering by amines should prove useful in understanding the role of clustering and internalisation in various cellular functions. Hormone actions which require internalisation should be blocked by amines, and actions which occur only as a result of surface binding should be unaffected or enhanced. Further studies of the amine inhibition of clustering should help to elucidate the molecular mechanism for the clustering of ligand-receptor complexes.

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Table 1 Genetic constitution of recipient-donor-target strain combinations of mice used to prepare alloantisera and Ia polypeptides

Strain	Haplo type	Regions of H-2									
		I								S	D
		K									
			A	B	J	E	C				
A. TH	(Recipient)	t2	s	s	s	s	s	s	s	s	d
A. TL	Donor	t1	s	k	k	k	k	k	k	k	d
B10. HTT	Target	t3	s	s	s	s	k	k	k	k	d
(B10×HTT) _{F1}	(Recipient)	b/i	b	b	b	b	b	b	b	b	b/d
B10. A (5R)	Donor	i5	b	b	b	k	k	d	d	d	d
B10. D2	Target	d	d	d	d	d	d	d	d	d	d

Boxed regions indicate the specificity of the antiserum against the target.

determine whether the N-terminal differences in the E_β polypeptides extend throughout other regions of these polypeptides and whether the E_α polypeptides are identical, we have undertaken tryptic peptide analyses of two Ia molecules from the I-E subregion. We report here the application of high-pressure liquid chromatography (HPLC) to the separation of tryptic peptides and present comparative tryptic peptide maps for I-E subregion products from the H-2^d and H-2^k haplotypes.

The procedures used for isolation and separation of the tryptic peptides of Ia polypeptides are described in Fig. 1. Table 1 gives the genetic constitutions of the mice used in the preparation of the alloantisera and the Ia antigens. The specificities of the antisera have been discussed elsewhere^{10,11}. Reverse-phase HPLC has been used to separate peptides of biological significance¹⁷ and tryptic peptides of globular proteins¹⁸. We now report its application to the separation of radiolabelled tryptic peptides of integral membrane proteins. We have found that the number of radiolabelled peptides which we detect by HPLC and conventional ion exchange, for both transplantation antigens (unpublished results) and Ia polypeptides, are comparable.

If the N-terminal homology observed between the E_α and E_β polypeptides and human Ia polypeptides continues throughout their entire structures, then from amino acid composition data for human Ia p29 (β) and p34 (α) polypeptides¹⁵, we can expect 23-26 tryptic peptides per molecule. Freed has reported ~12 tritiated arginine-containing peptides for the E_β polypeptide using conventional ion-exchange chromatography¹⁹. We detected about seven tritiated tyrosine-containing peptides and about 15 leucine-containing peptides for the E_β polypeptide (Fig. 1). Accordingly, we are monitoring approximately 30-65% (7/23-15/23) of the tryptic peptides of the polypeptide.

Comparison of tryptic peptides of the E_β polypeptides shows that there are several common peptides and multiple peptide differences (Fig. 1). We conclude that multiple amino acid differences are present in several regions of the E_α polypeptides. In contrast, the E_α^k and E_α^d polypeptides do not show any definite peptide differences (Fig. 1). One major peptide of the leucine map of the E_α^d polypeptide seems to be broader than its E_α^k counterpart; we cannot explain this difference. As comparative peptide maps overemphasise amino acid differences and as we cannot detect any definitive peptide differences, we conclude that the E_β polypeptides must be extremely similar, if not identical.

These comparative peptide maps permit us to draw several interesting genetic and evolutionary conclusions about the Ia polypeptides.

(1) Homology relationships. Several Ia polypeptides of the I-A subregion have been analysed by HPLC of tryptic digests¹². The E_α and A_α polypeptides share at most one tyrosine-containing peptide. The E_β and A_β polypeptides generally share only one or two peptides, although the A_β^b and E_β^k polypeptides show co-chromatography of four of seven tyrosine peptides. We do not know whether this apparent similarity is fortuitous or

Peptide map analyses of murine Ia antigens of the I-E subregion using HPLC

THE I REGION of the major histocompatibility complex (H-2) of the mouse¹ controls various immunologically related functions including immune responsiveness and T-cell, B-cell and macrophage interactions^{2,3}. The only gene products of the I region which have been identified by immunochemical techniques are the Ia antigens which are encoded by loci mapping to the I-A and I-E subregions of the I region⁴. Previously there has been ambiguity over whether the I-E and/or I-C subregion(s) control molecules detected by immunoprecipitation. Recent evidence suggests that the former, not the latter, controls these molecules^{5,6}. The Ia antigens are highly polymorphic by serological analysis and are expressed predominantly on B lymphocytes and epidermal cells⁷. Ia molecules are integral cell-surface glycoproteins and consist of two subunits of approximate molecular weights 35,000 (α) and 28,000 (β) which are noncovalently associated⁷. A general nomenclature has been agreed upon for the Ia polypeptides⁸. For example, the α polypeptide from the I-E subregion of a mouse of the H-2^d haplotype is denoted E_α^d . Partial N-terminal amino acid sequence analyses of Ia polypeptides from the I-E and I-A subregions of mice⁸⁻¹⁴, humans^{14,15} and guinea pigs¹⁶ have led to several important observations. (1) The E_α polypeptides of mice show striking homology with human α chains (p34) at least at their N-termini. The E_β polypeptides of mice also demonstrate some homology with their guinea pig and human counterparts. (2) The Ia polypeptides from the I-A subregion of mouse show little apparent homology with their human, guinea pig and I-E-subregion counterparts. (3) The A_α , A_β and E_β polypeptides of various haplotypes differ by one or more residues at their N-termini. However, for the E_α^d and E_α^k polypeptides no amino acid differences have been unequivocally demonstrated. To

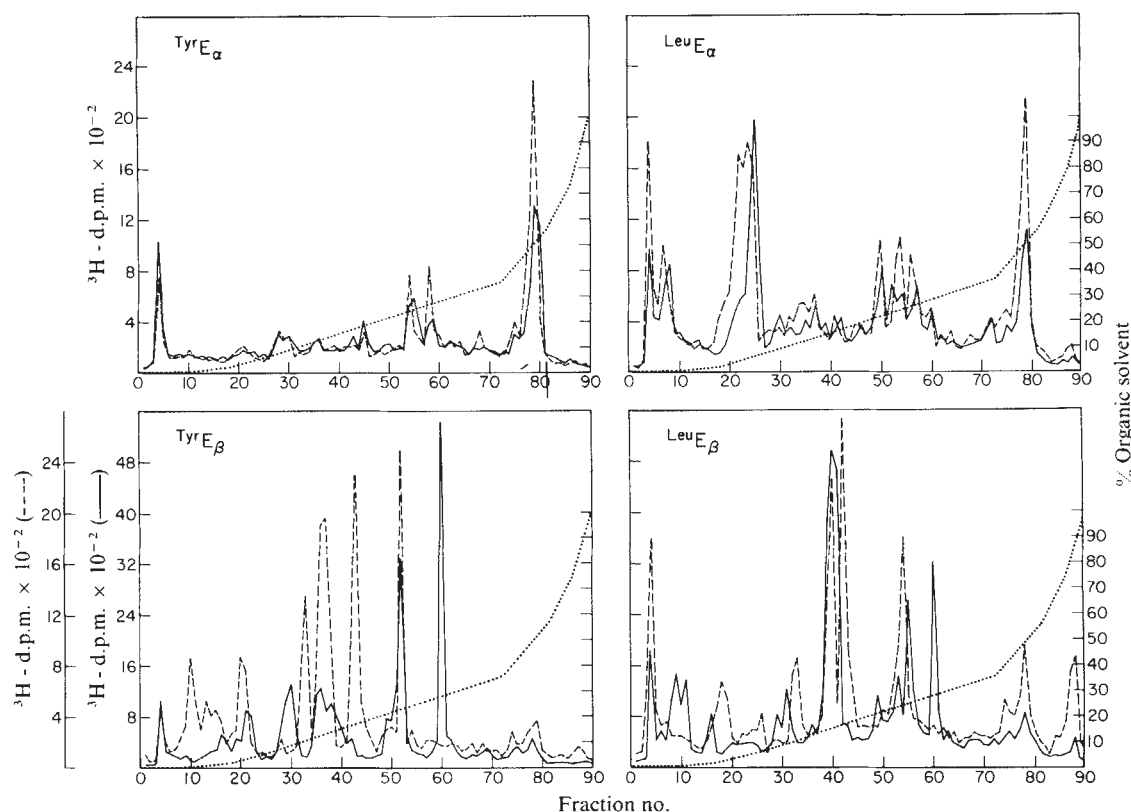


Fig. 1 HPLC tryptic peptide maps of α and β polypeptides of Ia molecules from the I-E subregion. —, Peptides of the k haplotype; ---, peptides of the d haplotype; ···, the concentration gradient of organic solvent used. Fraction number is plotted on the abscissa and the radioactivity of each fraction is plotted on the left ordinate. Ia antigens were biosynthetically radiolabelled and isolated by previously described procedures¹⁰. Splenic lymphocytes were cultured in the presence of either 1 mCi of tritiated tyrosine or 1 mCi of tritiated leucine. Radiolabelled Ia antigens were isolated by immunoprecipitation using specific alloantisera and *Staphylococcus aureus* Cowan I (ref. 23). The α and β polypeptides were separated by SDS-polyacrylamide electrophoresis²⁴ using 10% gels run in reducing conditions. The proteins were eluted from the gels in 0.01% SDS mixed with 0.5 mg porcine immunoglobulin and lyophilised. The polypeptides were reduced, alkylated and digested by the method of Brown²⁵ modified as follows. The polypeptides were boiled for 5 min in 0.5 ml of 0.6 M Tris, 0.02 M EDTA, 9% SDS, pH 8.3 buffer and reduced under argon for 3 h at 37 °C with dithiothreitol (0.025 M). The proteins were then alkylated in the dark for 1 h at room temperature using iodoacetamide (0.05 M) and reagents were then removed by centrifugation through a 2.5-ml column of Sephadex G-25 equilibrated with 0.05 M Tris, 2% 2-mercaptoethanol, 2% SDS, pH 7.5 (ref. 26). The proteins were then precipitated with 20% trichloroacetic acid, washed, dried and digested for 18 h at room temperature in 0.2 ml 0.05 M ammonium bicarbonate using 10 μ l of trypsin-L(tosylamide-2-phenyl)-ethyl-chloromethyl-ketone (Worthington) (10 mg ml⁻¹ in 0.001 M HCl). Another 10 μ l of trypsin solution was added and after 4 h the resulting peptides were lyophilised. The peptides were analysed on a DuPont 830 HPL chromatograph using a 25-cm Zorbax-CN column at 49 °C. The column was initially equilibrated with 0.1 M sodium phosphate buffer, pH 2.1, and the peptides were eluted with a gradient of acetone (Burdick and Jackson). 1-ml fractions were collected every minute directly into scintillation vials and evaporated to dryness. 0.5 ml 0.01% SDS and 4 ml Aquasol-2 (NEN) were added to each vial and the radioactivity was then determined on a Beckman LS9000 scintillation counter.

whether it signifies sequence homologies. In any case, it seems that the α and β polypeptides from the I-A and I-E subregions are not very closely related to one another.

(2) Genetic mapping. The mice from which the E^k and E^d molecules were isolated are congenic, presumably having distinct H-2 complexes superimposed on genetically identical B10 backgrounds. Haplotype-associated amino acid or peptide differences have been observed in the E_β polypeptides, which implies that the genes (structural or regulatory) controlling the synthesis of the E_β polypeptides are located within the H-2 complex. As the E_α gene product is apparently not polymorphic, no conclusions can be drawn about whether it lies in the H-2 complex. If the E_α^k and E_α^d polypeptides are indeed identical, the serological determinants on Ia molecules of the I-E subregion must reside on the corresponding E_β polypeptides.

(3) Complex allotypes. The E_β polypeptides exhibit multiple amino acid substitutions and can be defined as complex allotypes. The importance of distinguishing complex from simple allotypes, such as sickle-cell haemoglobin and its normal counterpart, is that a more complicated genetic organisation or evolutionary history is required to explain the generation of complex allotypes²⁰. The α and β polypeptides of the I-A subregion and the K and D transplantation antigens, and the β polypeptide of the I-E subregion are all examples of complex allotypes. Thus, five distinct genes (or gene systems) encoded by the H-2 complex exhibit the phenotypic behaviour of complex allotypes.

Jones *et al.*^{21,22} have reported that immunoprecipitates made using antisera having Ia specificities give a remarkably complex series of spots when analysed by two-dimensional gel electrophoresis and that these immunoprecipitates contain an invariant polypeptide of MW 31,000. We are now refining our peptide map procedures to identify which of these polypeptides have been analysed by amino acid sequence and peptide map techniques.

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Promotion of microtubule assembly *in vitro* by taxol

TAXOL (Fig. 1) was isolated from the plant *Taxus brevifolia* (western yew) by Wani *et al.*, who reported that the molecule has antitumour activity in several experimental systems¹. In our laboratory we have found that taxol, a low molecular weight neutral compound, completely inhibits division of exponentially growing HeLa cells at low concentrations of drug (0.25 μ M) that have no significant effects on DNA, RNA or protein synthesis during a 4-h incubation with the cells. HeLa cells incubated with taxol for 20 h are blocked in late G₂ and/or M (ref. 2). We report here that taxol acts as a promoter of calf brain microtubule assembly *in vitro*, in contrast to plant products such as colchicine and podophyllotoxin, which inhibit assembly. Taxol decreases the lag time for microtubule assembly and shifts the equilibrium for assembly in favour of the microtubule, thereby decreasing the critical concentration of tubulin required for assembly. Microtubules polymerised in the presence of taxol are resistant to depolymerisation by cold (4 °C) and CaCl₂ (4 mM).

Many models have been proposed for the microtubule assembly system used in these experiments³, but the exact mechanism for microtubule assembly *in vitro* is not known; the conditions required have been described elsewhere⁴. A dynamic equilibrium of microtubules with tubulin dimers has been demonstrated *in vitro*^{5–7}. Our standard conditions for assembly in a final volume of 1.0 ml at 37 °C are: 1 mM EGTA, 0.5 mM MgCl₂, 1 mM GTP, 0.1 M 2-[N-morpholino]ethane sulphonic acid (MES) at pH 6.6 and 1 mg ml⁻¹ tubulin.

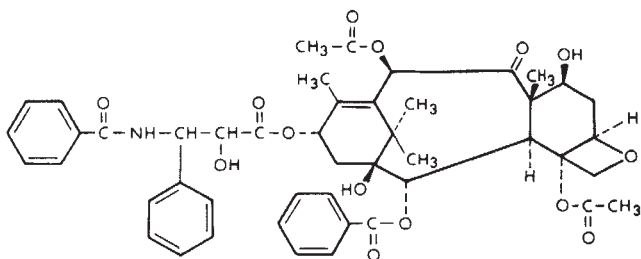


Fig. 1 Structural formula of taxol.

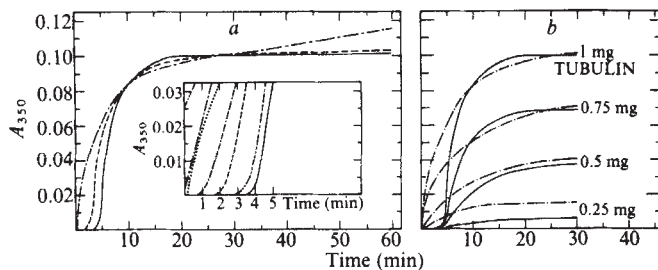


Fig. 2 Effect of taxol on microtubule assembly kinetics. Calf brain tubulin was prepared by two cycles of assembly–disassembly¹⁹ and stored at –20 °C in MES buffer (0.1 M MES, 1 mM EGTA, 0.5 mM MgCl₂, adjusted to pH 6.6 with NaOH) containing 1 mM GTP and 4 M glycerol. Before each experiment the tubulin was dialysed for 3 h at 4 °C against 100 vols of MES buffer, and centrifuged at 120,000g for 20 min at 4 °C. Tubulin present in the supernatant was maintained in an ice bath. Tubulin purified by this method is 85% pure as determined on 3–27% SDS–polyacrylamide gradient reducing slab gels²⁰. Assembly was monitored by the method of Gaskin *et al.*⁵. The cuvettes (1 cm path) containing MES buffer, drug and 1 mM GTP were kept at room temperature before addition of tubulin and shifting to 37 °C. Turbidity measurements were made at 350 nm every 20 s on a Gilford spectrophotometer equipped with an automatic recorder and a thermostatically regulated sample chamber. Taxol was dissolved in dimethyl sulphoxide at 10 mM and stored at –20 °C. The final concentration of dimethyl sulphoxide (0.5%) had no detectable effect on the assembly of tubulin. *a*, Standard assembly conditions: no additions (—); 0.05 (---); 0.1 (---); 0.5 (---); 1 (---); 5 (---); and 10 (---) μ M taxol. *b*, Standard assembly conditions: 0.25, 0.5, 0.75 or 1 mg ml⁻¹ tubulin, 37 °C. No additions (—); 5 μ M taxol (---).

Microtubule assembly was monitored by the increase in turbidity⁵ and by electron microscopy. In our conditions, taxol causes a dose-dependent decrease in the lag time for assembly (Fig. 2a); whereas the control has a 3.5 min lag time, 5 μ M taxol essentially eliminates the lag time. The control assembly progress curve is sigmoid, but addition of 5 μ M taxol gives hyperbolic kinetics. Ribbon structures and dimers are seen with the electron microscope at 30 s with 1 mg ml⁻¹ tubulin and 5 μ M taxol; in the control there are only rings and dimers (data not shown). The ribbon structures observed at 30 s with taxol are probably intermediates in assembly, as only microtubules are seen at 30 min. Figure 2b demonstrates that taxol decreases the lag time independently of tubulin concentration.

Microtubules polymerised in the presence of 10 μ M taxol are shorter than in the control. Average microtubule lengths were determined by electron microscopy⁷ at 30 min, a time at which equilibrium was apparently reached. The average length of the control microtubules is $4.12 \pm 2.12 \mu$ m, whereas the microtubules polymerised in the presence of 10 μ M taxol have an average length of $1.49 \pm 0.65 \mu$ m.

Microtubules were polymerised in the absence and presence of 5 μ M taxol for 30 min at 37 °C, and then chilled in an ice bath for 30 min. By electron microscopy, microtubules were seen only in the drug-treated sample. Ribbon structures, but no microtubules, were observed when polymerisation was carried out at 4 °C for 30 min in the presence of 50 μ M taxol. Microtubules assembled at 37 °C in the presence of 10 μ M taxol were completely resistant and those assembled in the presence of 5 μ M taxol were partially resistant to depolymerisation by 4 mM CaCl₂ (Fig. 3a). Dimers, rings and microtubules were present 30 min after the addition of CaCl₂ to microtubules assembled in the presence of 5 μ M taxol. Figure 3b demonstrates that microtubule assembly will take place in the presence of 4 mM CaCl₂ and 5 μ M taxol.

Microtubules were centrifuged at 120,000g for 30 min and resuspended in 1 ml of MES buffer with or without 10 μ M taxol. After 4 min, 4 mM CaCl₂ was added to the resuspended samples. Continuous turbidity measurements and electron microscopy indicated that no microtubules were present in the non drug-treated sample after the addition of CaCl₂, whereas

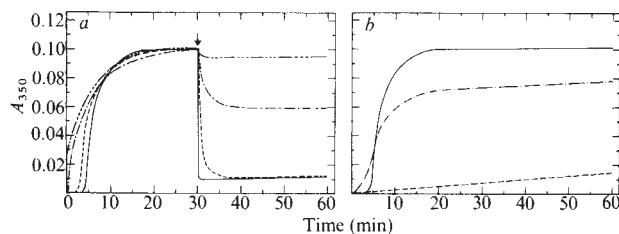
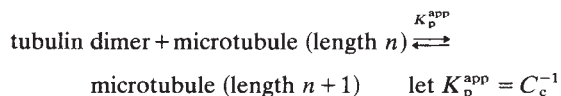


Fig. 3 Effect of CaCl_2 on microtubules assembled *in vitro* in the presence of taxol. *a*, Standard assembly conditions. No additions (—); 0.1 (---); 5 (···); and 10 (— · —) μM taxol. CaCl_2 is added at a final concentration of 4 mM at 30 min (↓). *b*, Assembly conditions as in (*a*). No additions (—); 4 mM CaCl_2 plus 5 μM taxol (---); 4 mM CaCl_2 (···).

the taxol-treated microtubules were resistant to depolymerisation by CaCl_2 . Such experiments suggest that a taxol binding site is available on the intact microtubule.

Tubulin (7.7 μM ; 1 mg ml^{-1} total protein) was assembled in standard conditions in the absence and presence of 10 μM taxol for 20 min. After centrifugation at 120,000g for 30 min, the amount of protein in the pellets and supernatants was determined⁸. In the absence of taxol, 0.57 mg of protein was pelleted by centrifugation. When tubulin was assembled in the presence of 10 μM taxol, 0.78 mg of protein was pelleted. A maximum amount of protein was pelleted at a taxol concentration approximately stoichiometric with the dimer concentration.

There is evidence that microtubule assembly *in vitro* takes place by the nucleated condensation mechanism of Oosawa and Kasai⁹. This model is supported by a requirement for a critical concentration (C_c) of protein to achieve assembly, and by tubulin-microtubule equilibrium data^{5,6,10}. The critical concentration is approximately the inverse of the apparent microtubule propagation equilibrium constant (K_p^{app}) for the addition of a single tubulin dimer to a pre-existing microtubule⁵.



Below the critical concentration, no microtubules are found by light scattering, electron microscopy or sedimentation velocity^{5,10,11}.

Taxol causes a decrease in the critical concentration of protein required for microtubule assembly *in vitro* (Fig. 4). Protein concentration was determined in both the supernatants and pellets. In the absence of drug (Fig. 4*a*), the critical concentration observed is 0.2 mg ml^{-1} (1.8 μM), a value which corresponds well with that previously reported⁵. In the presence of 5 μM taxol (Fig. 4*b*) the critical concentration decreased to

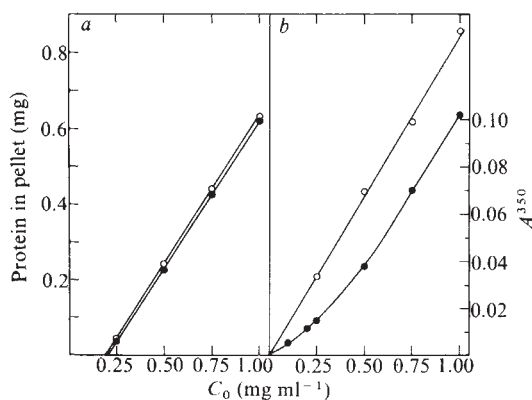


Fig. 4 Comparison of critical concentrations determined by centrifugation (120,000g at 25 °C for 30 min) of microtubules (○) and turbidity measurements (●) in the absence (*a*) and presence (*b*) of 5 μM taxol. C_0 is the total protein concentration. Determinations are made after a 30 min incubation in standard assembly conditions.

$\leq 0.01 \text{ mg ml}^{-1}$ (0.08 μM). The values for the apparent standard free energy ($\Delta G_{\text{app}}^\circ$) of addition of a tubulin dimer to a growing microtubule have been calculated using the formula $\Delta G_{\text{app}}^\circ = -RT \ln K_p^{\text{app}}$. For control and taxol-treated (5 μM) preparations, respectively, values for K_p^{app} were 5.7×10^5 and $\geq 1.1 \times 10^7 \text{ l mol}^{-1}$, and for $\Delta G_{\text{app}}^\circ$, -8.0 and $\leq -10.0 \text{ kcal mol}^{-1}$. As taxol renders microtubules resistant to depolymerisation, the change in the apparent equilibrium constant could be the result of a reduced dissociation rate constant.

Turbidity at 30 min did not change appreciably compared with that of the control until the protein concentration was less than 0.25 mg ml^{-1} (Figs 2*b*, 4). Microtubules, tubulin hoops¹² with less than 13 protofilaments, and ribbons were observed at 0.1 mg ml^{-1} tubulin in the presence of 5 μM taxol at 30 min, whereas the control contained no structures (Fig. 5). At 1 mg ml^{-1} tubulin and 5 μM taxol, essentially no hoop structures or ribbons, only short microtubules, were found at 30 min.

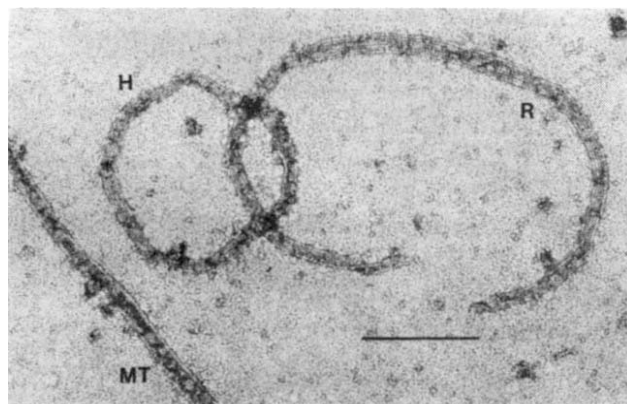


Fig. 5 Electronmicrograph of the structures assembled in the presence of 0.1 mg ml^{-1} tubulin and 5 μM taxol at 30 min in standard conditions. Structures were negatively stained with uranyl acetate²¹. MT, Microtubule; H, hoop; R, ribbon. Scale bar, 0.2 μm .

More and shorter microtubules are polymerised when assembly takes place in the presence of taxol. These shorter microtubules do not scatter light to the same degree as the control microtubules at the identical protein concentration⁵. However, as there are more of the short microtubules than there are of the control microtubules, there is very little difference in the amount of turbidity that develops at concentrations of protein above 0.25 mg ml^{-1} at 30 min. If the average length of the microtubules and the mass of protein pelleted after polymerisation in the presence of 10 μM taxol are taken into account, there is an approximate 3.8-fold increase in the number of microtubules (nucleations) in the drug-treated sample.

Phosphocellulose-purified tubulin, which is free of microtubule-associated proteins¹³, will assemble in the presence of stoichiometric concentrations of taxol. Taxol also promotes assembly of charcoal-treated tubulin (1 mg ml^{-1}), which contains substoichiometric concentrations of GDP at the exchangeable site¹⁴. The drug has no effect on GTP or GDP hydrolysis in our microtubule preparations (data not shown).

There are similarities between microtubule and F-actin polymerisation. A nucleated condensation mechanism for the polymerisation of F-actin has previously been described¹⁵. Opposite-end assembly and disassembly have been shown for both F-actin polymerisation¹⁶ and microtubule polymerisation at steady state *in vitro*¹⁷. Bound nucleoside triphosphate is hydrolysed during polymerisation in both systems. Phalloidin, a bicyclic peptide from the toxic mushroom *Amanita phalloides*, has been reported to accelerate the polymerisation of F-actin and confer resistance to depolymerisation¹⁸. Phalloidin and taxol represent natural products that enhance self-assembly systems. From our results, we consider that there may be low molecular weight cell constituents with activity similar to that of

taxol which may be involved in the regulation of microtubule assembly in the cell.

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Molecular basis of thermostability in the lysozyme from bacteriophage T4

MOST proteins are denatured at temperatures above 50-60 °C, although some enzymes, especially those from thermophilic organisms, remain active at temperatures up to 80-90 °C. The determination of the three-dimensional structure of the thermostable protease thermolysin showed that heat-stable proteins do not contain unusual structural features absent from less stable proteins^{1,2}. Furthermore, the amino acid sequences of similar proteins from both mesophilic and thermophilic sources have been shown to be homologous, suggesting that the respective structures are similar^{3,4}. Nevertheless, such homologous amino acid sequences also include many differences which obscure those amino acid changes actually responsible for differences in thermostability. We report here the structure of a temperature sensitive (ts) mutant of T4 phage lysozyme. This permits the first direct comparison of two protein structures in which all differences are directly related to a change in thermal stability. It is shown that, except for the replacement of a partially exposed arginine by a histidine, the three-dimensional structure of the ts lysozyme is virtually identical with that of native lysozyme.

Bacteriophage T4 lysozyme, an endoacetylmuramidase, is produced late in the infection of *Escherichia coli* by T4

bacteriophage. It cleaves the bacterial cell wall, allowing the release of the progeny phage particles⁵. The amino acid sequence of the lysozyme has been determined⁶, crystals suitable for detailed X-ray study have been obtained, the three-dimensional structure determined^{7,8} to a resolution of 2.4 Å, and a thermodynamic analysis of the folding of the enzyme is underway (ref. 9 and unpublished results).

Since Streisinger developed a technique for identifying and isolating phage particles with mutations in the lysozyme gene¹⁰, many different mutations in the lysozyme gene have been identified, the resultant changes in the amino acid sequence of the protein determined, and the properties of these mutant lysozymes discussed in the light of the three-dimensional structure of the wild-type enzyme^{8,11}.

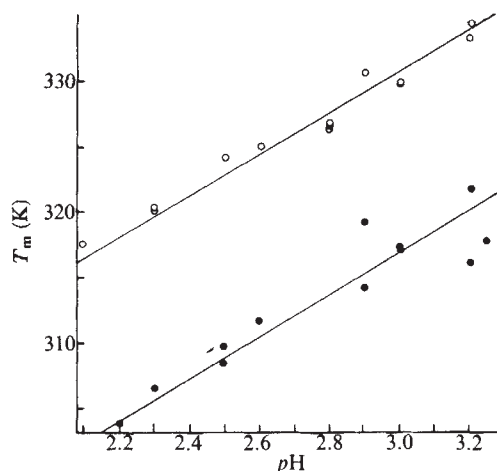


Fig. 1 The melting temperature (T_m) of wild-type (○) and temperature-sensitive (●) lysozymes as a function of pH. Denaturation was monitored by following changes in peptide circular dichroism at 223 nm. Details of the experimental method and of the analysis used to calculate T_m are given elsewhere⁹. Solvent conditions were as follows: 0.2 M NaCl, 10^{-4} M dithiothreitol, 10^{-6} - 10^{-5} M lysozyme.

Lysozyme mutant strains were generated by use of 2-aminopurine, which induces single base pair substitutions. Temperature-sensitive mutants were selected applying the recognition method for lysozyme gene mutations¹⁰. Plaques of wild-type phage on plates incubated at 37 °C become surrounded by large haloes after exposing the plate to chloroform vapour. The haloes result from phage lysozyme diffusing from the plaque into the surrounding area and lysing the chloroform-treated bacteria. Halo size is a direct measure of the stability of the lysozyme. For ts mutants, the halo size decreases with increasing temperature on plates incubated at 30, 37 and 41 °C whereas wild-type plaque haloes stay large. By testing the ability of the mutant phage to recombine with a number of precisely mapped deletion mutants in the lysozyme gene, the locus of the amino acid change was determined to lie between residues 65 and 105.

The ts mutant lysozyme was purified according to the standard procedure for wild-type lysozyme¹², and was found to have a melting temperature about 14° lower than that of the native enzyme (Fig. 1). Crystals isomorphous with the native enzyme were obtained by the standard procedure⁸. The largest cell dimension change, in *c*, was 0.4 Å. We therefore were able to analyse the mutant by the standard difference Fourier technique (for example see ref. 13). A data set to 2.4 Å resolution was collected and a difference electron density map calculated between the native and the mutant lysozyme. Full details of the structure determination will be published elsewhere.

The difference density map is essentially featureless except at the location of the side chain of Arg 96, where a strong negative feature is seen at the site of the guanidinium group, and a positive peak overlaps the positions of C^γ and C^δ (Fig. 2). Clearly, Arg 96 in wild-type lysozyme has been replaced in the ts

mutant by an amino acid with a medium-size side chain. The location of the substitution is consistent with that determined genetically. Determination of the amino acid composition of the mutant lysozyme revealed that there were two histidines, compared with one in the wild-type enzyme, indicating that Arg 96 has been replaced by a histidine. Furthermore, the electron density map (Fig. 2) also strongly suggests that the ts lysozyme has a histidine at position 96.

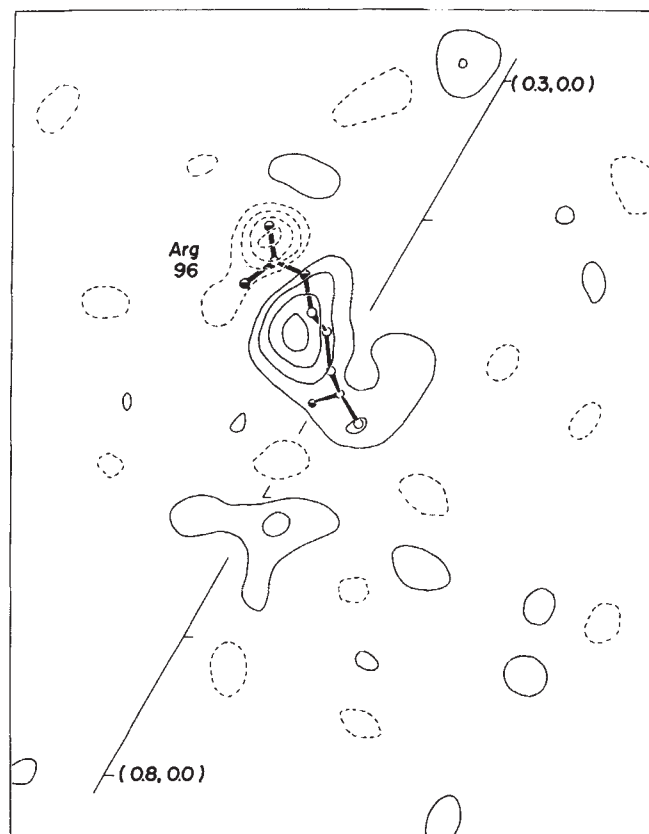


Fig. 2 Portion of the map showing the difference in electron density between native phage lysozyme and the temperature-sensitive mutant lysozyme with Arg 96 replaced by a histidine. The section shown, $z = 11/132$, approximately intersects the side chain of Arg 96. Contours are drawn at arbitrary equal intervals, with positive contours solid and negative broken. In the superimposed side chain of Arg 96, carbon atoms are represented by open circles and nitrogen by half solid circles.

In the native enzyme, the side chain of Arg 96 is well-ordered, and lies on the protein surface between the ring of Tyr 88 and the main chain and C^β of Leu 91 (Fig. 3). The hydrocarbon portion of the arginine side chain contributes to the major hydrophobic core of the C-terminal lobe of the molecule^{7,8}. Substitution of an imidazole at this position presumably destabilises the hydrophobic core somewhat, providing at least part of the molecular basis for the observed temperature sensitivity. The imidazole ring of His 96 lies parallel to the ring of Tyr 88, and appears to donate a hydrogen bond to the carbonyl oxygen of the same residue (Fig. 3), possibly destabilising the α -helix 82–90. Detailed analysis of these interactions will take some time.

The main finding, however, which appears clearcut, is that the three-dimensional structure of the ts lysozyme is virtually identical with that of native lysozyme, except for the replacement of a partially exposed arginine by a histidine. Excluding residue 96, there is no evidence for structural changes larger than a few tenths of an angstrom. This finding is consistent with our earlier suggestion^{2,14}, based on the structure of the thermostable protease thermolysin, that differences in the thermostability of

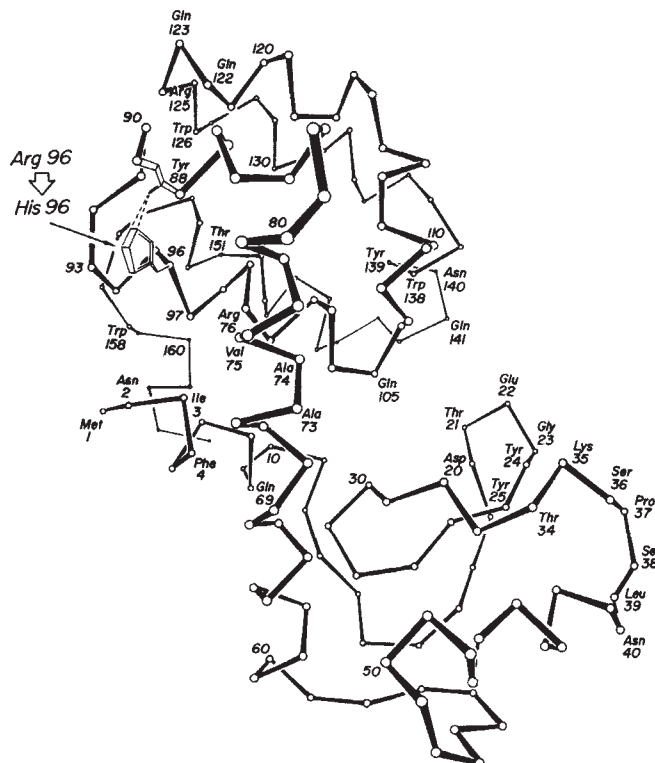


Fig. 3 Schematic drawing of the backbone of bacteriophage T4 lysozyme showing the location of His 96 in the temperature-sensitive mutant. (Adapted from ref. 8).

proteins are, in general, due to subtle changes in hydrophobic interactions, hydrogen bonds, and so on, and not to a single determinant such as metal binding or changes in secondary structure. In agreement with Perutz¹⁵, our study shows that extra energy of thermal stabilisation can be provided without disturbance of the tertiary structure of the protein, although we do not see any change in the number of salt bridges in the mutant lysozyme. Also, our results confirm general thermodynamic arguments which suggest that the net free energy of stabilisation of proteins is small, and derives from a delicate balance between large stabilising forces, principally due to hydrophobic interactions, and large destabilising ones, principally due to chain entropy^{16–18}.

In addition to the mutant described here, three other temperature sensitive mutant lysozymes, with amino acid substitutions in other parts of the molecule, have been crystallised isomorphously with the wild-type enzyme. Clearly, these mutant lysozymes also have structures similar to that of the native enzyme. We hope that structural comparisons of these and other modified lysozymes will eventually provide a detailed accounting of the contributions of individual amino acid residues to the stability of a protein. Related studies of mutant lysozymes with modified activity should help clarify the mechanism of action of the enzyme.

We thank Kerrie Rine for technical assistance; Drs G. Streisinger and J. Owen for advice on lysozyme genetics, and Dr J. Schellman for discussions on lysozyme stability. M.G.G. was supported by the Swiss NSF. This work was supported in part by NIH grants GM 21967, GM 20066 and GM 20195.

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Effect of a local anaesthetic on hydrocarbon chain order in membranes

THE mode of action of anaesthetics has provoked considerable interest and speculation^{1,2}. For tetrodotoxin³, it is clear that direct interaction of the anaesthetic with the sodium channel blocks nerve conduction; in other cases changes in lipid bilayer structure, such as membrane expansion², fluidus expansion¹ or surface-charge modulation⁴, have been invoked. Recently, a mechanism involving an increase in lipid bilayer thickness has been suggested from results obtained using black film techniques^{5–7}. We have investigated the hydrocarbon chain length of a lecithin bilayer in the presence and absence of the anaesthetic benzyl alcohol^{8,9} using high-field deuterium nuclear magnetic resonance (NMR) spectroscopy of specifically deuterated lipids^{10–16}, to determine the changes in bilayer structure that occur on addition of the anaesthetic alcohol. We report here that at concentrations used for local anaesthesia there is no change in membrane thickness. By comparison, cholesterol (at 0.3 mol fraction) causes an ~0.46 nm increase in membrane thickness. We therefore suggest that the amount of solvent (tetradecane) in black lipid membranes, and hence their thickness, may be influenced by the presence of benzyl alcohol and cholesterol⁵.

Figure 1a shows the effect of benzyl alcohol on the deuterium quadrupole splitting of a dimyristoyl lecithin labelled as ²H₂ in the sixth position of the 2-chain. As the concentration of benzyl alcohol increases, the quadrupole splitting and therefore the order parameter of the labelled methylene segment¹³ decreases. This suggests a decrease in order of the 2-chain; cholesterol causes an increase^{10,11,15,16}. Determination of the change in order parameter at each segment in the hydrocarbon chain permits evaluation of the total change in hydrocarbon chain length^{10,13,17}. We determined the quadrupole splittings, and the order parameters, for a range of dimyristoylphosphatidylcholines labelled (as C²H₂ or C²H₃) at one of positions C-2, C-3, C-4, C-6, C-8, C-10, C-12, or C-14, in the 2-chain, at a total lipid-anaesthetic mol ratio of 1:3. In Fig. 1b representative spectra illustrate the effect of benzyl alcohol on the deuterium quadrupole splittings of a series of ²H-labelled phosphatidylcholines, and Fig. 1c shows the effects produced by benzyl alcohol and cholesterol on these splittings. Benzyl alcohol and cholesterol have opposite effects on the order of the phosphatidylcholine acyl chains. Benzyl alcohol (above the gel-liquid crystal phase transition temperature of the phospholipid) causes a decrease in hydrocarbon chain quadrupole splitting or order parameter; cholesterol (above the gel-liquid crystal phase transition temperature of the phospholipid) causes an increase in hydrocarbon chain quadrupole splitting, or order

parameters. Qualitatively, therefore, benzyl alcohol decreases and cholesterol increases hydrocarbon chain order.

At anaesthetic concentration, however, there is no effect of benzyl alcohol on membrane thickness. Local anaesthetic concentrations of benzyl alcohol are 4 mM for rat phrenic nerve and 12 mM for frog sciatic nerve⁹. At 40 °C the partition coefficient of benzyl alcohol between multi-bilayers of dimyristoyl

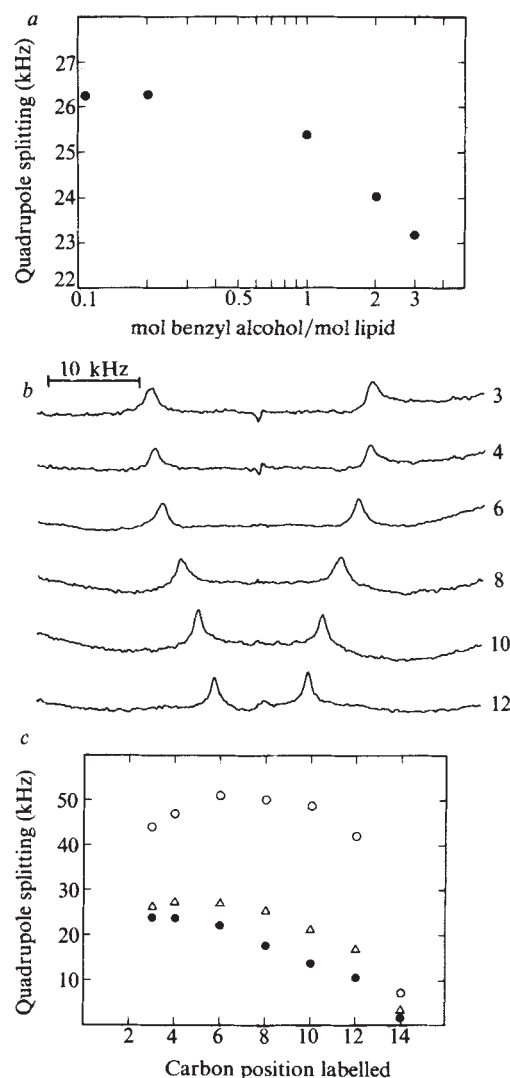


Fig. 1 Effects of benzyl alcohol, and of cholesterol, on deuterium NMR quadrupole splittings of specifically ²H-labelled dimyristoylphosphatidylcholines. *a*, Graph of the quadrupole splitting of 2-(6', 6'-d₂)dimyristoylphosphatidylcholine (10 mg) dispersed in excess deuterium-depleted water (250 μl) containing benzyl alcohol at 38 °C, as a function of the benzyl alcohol-lipid mol ratio. The pure lipid quadrupole splitting value is 26.2 ± 0.5 kHz. *b*, Representative deuterium Fourier transform NMR spectra of dimyristoylphosphatidylcholines, labelled in the 2-chain as C²H₂ at the positions indicated. The samples contained a benzyl alcohol-lipid mol ratio of 3:1; spectra were recorded at 38 °C. *c*, Graph of quadrupole splittings of specifically deuterated dimyristoylphosphatidylcholines as a function of position labelled in the hydrocarbon chain, in the presence and absence of benzyl alcohol (3:1 benzyl alcohol-lipid mol ratio) or cholesterol (30 mol % cholesterol). Pure lipid and lipid-benzyl alcohol data obtained at 38 °C, lipid-cholesterol data obtained at 30 °C. Deuterium NMR spectra were obtained using the Fourier transform method at 5.2 T (corresponding to a deuterium resonance frequency of 34 MHz) on a homebuilt instrument¹⁰. The synthesis of dimyristoylphosphatidylcholines specifically deuterated in the 2-chain are described elsewhere¹⁰. Benzyl alcohol was added as an aqueous solution to dry phospholipid and the mixtures homogenised at about 40 °C. Δ, Dimyristoylphosphatidylcholine alone; ○, in the presence of cholesterol; ●, in the presence of benzyl alcohol.

lecithin and water is 13.9 according to Katz and Diamond¹⁸. At local anaesthetic levels, the benzyl alcohol-dimyristoyl lecithin mol ratio is 0.04–0.11. As may be seen from Fig. 1a there is no decrease in the electric quadrupole splitting from the pure lipid value of 26.2 ± 0.5 kHz in this range of benzyl alcohol concentration, a result consistent with spin-label studies⁹, although on a different timescale and without the possible complications of a perturbing probe effect.

At higher benzyl alcohol-dimyristoyl lecithin mol ratios there is a decrease in quadrupole splitting (or order parameter) with increasing concentration of benzyl alcohol, which agrees with spin-label studies⁹ (Fig. 1). From these results it is relatively straightforward to determine quantitatively the projections of each C–C segment onto the bilayer normal^{10,13,17}, and so determine the effective length of the hydrocarbon chain, or the total membrane thickness^{10,13,17,19}. Calculated chain lengths are rather insensitive to chain tilt. For example, for pure dimyristoylphosphatidylcholine at 60 °C, the distance from C-2 to C-14 is 10.2(6) Å assuming no chain tilt, or 10.5(2) Å assuming a gaussian distribution of chain orientations with a probable tilt angle of 25° (refs 10, 17). Similar average chain lengths are obtained using flat or lorentzian distribution functions (E.O. and R. Jacobs, unpublished). Consequently, we obtain from the NMR data a model-insensitive value for the average chain length, or membrane thickness. These lengths are in excellent agreement with those determined using high-resolution neutron diffraction (D. Worcester, M. Meadows, D. Rice and E.O., unpublished). For example, 23 °C lecithin-cholesterol data give a total hydrocarbon region thickness of about 30–31 Å, and neutron diffraction measurements¹⁰ indicate a thickness of about 33 Å (Table III of ref. 10). From the data in Fig. 1b and c we can calculate the thickness of the dimyristoylphosphatidylcholine membrane at 38 °C and obtain values of about 2.5(0) nm for the hydrocarbon region thickness in the absence of benzyl alcohol and about 2.3(6) nm in the presence of benzyl alcohol. The fluidisation of the bilayer caused by benzyl alcohol^{8,9} decreases the membrane thickness. Cholesterol (30 mol%) causes an increase in hydrocarbon chain order, corresponding to about a 0.46-nm increase in membrane thickness at 23 °C (ref. 10).

Previous workers^{5,6} have found, using black lipid membrane capacitance and conductance measurements, a 1.2-nm increase in membrane thickness in the presence of benzyl alcohol, and a decrease in hydrocarbon region thickness in the presence of cholesterol. Although the fatty acid composition of the lecithin used in the black lipid membrane studies differed slightly from ours, neutron beam and X-ray diffraction measurements on the egg-lecithin-cholesterol system indicate no significant decrease in membrane thickness on addition of cholesterol^{21–23}. Similarly, other ²H NMR and electron spin resonance spin-label studies of egg lecithin and egg-lecithin-cholesterol systems indicate an ordering of the hydrocarbon chains by cholesterol^{15,24,25}, which must correspond to an increase in projected chain length. In the absence of hydrocarbon solvents, such as tetradecane, an increase in projected chain length must correspond to an increase in membrane thickness, as neutron and X-ray data rule out ≥ 0.2 nm-chain interdigitation.

We therefore suggest that the previous black lipid film results^{5,6} may be attributable to the inclusion of variable concentrations of solvent tetradecane in the lipid membrane, the exact amount being a function of the amount of cholesterol or benzyl alcohol present.

Deuterium nuclear magnetic resonance results suggest that the direct effect of benzyl alcohol on membrane bilayer thickness is negligible at the anaesthetic levels commonly used in clinical studies. At much higher levels (corresponding to an ~30–80-fold overload) there are small (≈ 0.1 nm) decreases in membrane thickness which are consistent with recent X-ray and neutron-beam studies of the effects of gaseous general anaesthetics on lipid bilayer thickness²⁶. Taken together, our results cast considerable doubt on the importance of membrane thick-

ness changes as a factor in the mechanism of action of many anaesthetics.

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X-ray diffraction patterns from molecular arrangements with 38-nm periodicities around muscle thin filaments

A SERIES of meridional and near-meridional reflections have been observed in X-ray diffraction patterns (ref. 1 and Fig. 1a) from the crab *Plagusia* leg muscle in the living, resting state, and have been attributed to troponin molecules which lie in pairs on thin filaments at 38-nm intervals. In the pattern (ref. 1 and Fig. 1b) obtained from the same muscle in rigor, the corresponding series of reflections was observed to have stronger intensities and higher order reflections. The reflections were interpreted as arising from both troponin and the cross-bridges. Electron micrograph² showed that cross-bridges occurred in symmetrical pairs around the thin filaments with a repeating distance of 38 nm in which one or a few closely spaced pairs may be included. Recently, reflections indexed as orders of 76 nm in X-ray diffraction patterns from insect flight muscle in rigor³ and those from scallop striated muscle in rigor⁴ have also been interpreted in terms of the cross-bridges attached in pairs to thin filaments with a repeating distance of 38 nm. We report here a method that enables us to analyse diffractions generated by molecular arrangements of this type. These arrangements are described by a set of identical helices, and the systematic modulation of intensities, indexed as orders of 76 nm, is interpreted in terms of displacement between these helices.

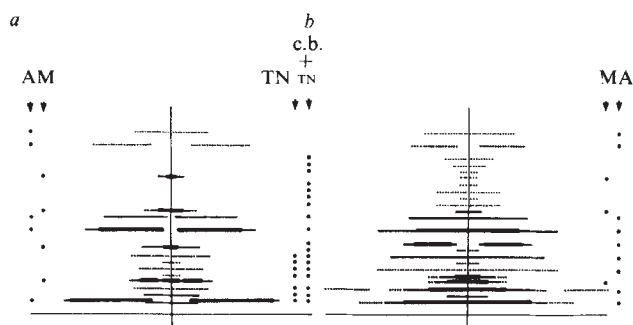


Fig. 1 Schematic representation of X-ray diffraction patterns¹ from crab *Plagusia* leg muscle in the living resting muscle (a), and in rigor (b). In addition to actin layer lines (marked by A) and meridional reflections (marked by M) attributed to myosin projections on the surface of the thick filaments, there is a series of meridional and near-meridional reflections (including intensities observed at $<0.07 \text{ nm}^{-1}$ on some of the actin layer lines) which were indexed as orders of $2 \times 38 \text{ nm}$. In a, the reflections (marked by TN) were seen up to the 9th order, whereas in b those (marked by c.b. + TN) were observed up to the 24th. Thick solid lines, solid lines and dotted lines represent intensities observed even on the third film, up to the second one and on only the first film of each set, respectively. Bar, 0.1 nm^{-1} .

The thin filaments consist of actin, tropomyosin and troponin in a molar ratio of 7:1:1. The actin helix has a symmetry of 28/13 (28 molecules are included in 13 turns of the short-pitch helix) in the crab leg muscle in rigor. If we assume that these protein molecules interact with each other at specific sites, then the arrangement of troponin can be described by two identical 2/1 helices as shown in Fig. 2a. Axial displacement (Δz) and azimuthal rotation ($\Delta\phi$) between two molecules in pairs are specified by k multiple of those values between adjacent two-actin subunits along the 28/13 actin helix, where k is an odd integer. Ohtsuki^{5,6} proposed a plausible model with $k = 1$, which remains to be proved.

Uniformly spaced layer lines, indexed as orders of 76 nm, are predicted from the single 2/1 helix as shown in Fig. 3a. Every even (odd) order Bessel function contributes to every even (odd) order layer line. The addition of another 2/1 helix results in a systematic intensity modulation which depends on k . As there is no trace of lattice sampling on the layer lines in the actual resting and rigor patterns from the crab leg muscle, we propose that the cylindrically averaged intensity⁷ on each layer line can be written as

$$\langle F(R)F^*(R) \rangle_l = \sum_n J_n^2(2\pi Rr) |a_l^n|^2 \quad (1a)$$

$$a_l^n = \sum_j \exp i\{-n\phi_j + 2\pi lz_j/c\} \quad (1b)$$

where J_n is the n th order Bessel function, a_l^n the modulation term, and r , ϕ_j and z_j radial, azimuthal and axial coordinates of a molecule on the j th helix. In equation (1a), the effect of shape and size of a subunit on the intensity is not included. The summation in equation (1a) is taken over n , satisfying the selection rule⁸ of $l = n + 2m$, where m is an integer. As $\Delta\phi = (1 - 1/14)\pi k$ and $\Delta z = c/28 \times k$, then $|a_l^n|^2 = 4\cos^2\{\pi k/28 \times (l + n)\}$ for $l = \text{even}$ and $4\sin^2\{\pi k/28 \times (l + n)\}$ for $l = \text{odd}$. If $k = 1$ is assumed, even order Bessel terms would be dominant around the equator and $l = 28$, and odd orders would be dominant around $l = 13$ and 15 as shown in Fig. 3c, because modulation terms for meridional J_0 reflections (near-meridional $J_1 + J_{-1}$ reflections), for example, gradually vary with even (odd) values of l . If $k = 3$ is assumed, modulation terms vary three times faster with l (Fig. 3d).

In the actual resting pattern from the crab leg muscle (ref. 1 and Fig. 1a), (1) reflections are observed at every order from 2nd to 9th, except at 5th, (2) even order reflections have intensity maxima on the meridian whereas odd orders have not.

These features are well explained only when $k = 1$. The presence of a meridional reflection at $l = 4$ and near-meridional one at $l = 9$ are hardly explained by $k = 3$. $k = 5$ and 7 are also excluded. The near meridional reflection at $l = 5$ may be buried in the strong reflection at 14.4 nm (assigned to thick filament). In the muscle in the resting state, the actin helix symmetry is slightly different from 28/13, and accordingly, the troponin

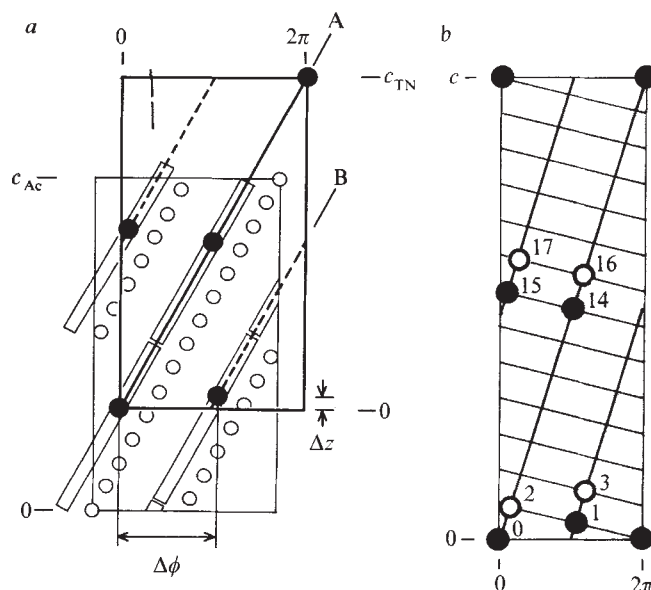


Fig. 2 a, The radial projection of the thin filament in Ohtsuki's model. A troponin molecule (closed circle) is attached to a specific site on a tropomyosin molecule (rectangular) which interacts specifically with seven actin subunits (open circles) arranged in a helix with the symmetry of 28/13 and the repeating distance of c_{Ac} . The arrangement of troponin molecules consists of two identical 2/1 helices, A and B, with the repeating distance of $c_{TN} = c_{Ac}$. Axial displacement (Δz) and azimuthal rotation ($\Delta\phi$) between two helices are specified by k multiples of those values between adjacent two-actin subunits along the 28/13 actin helix, where k is an odd integer. Ohtsuki's model assumed $k = 1$. In the crab leg muscle¹, the symmetry of the actin helix is 28/13 in rigor, but in the living resting state it is between 26/12 ($= 13/6$) and 28/13. This means that the troponin helix differs slightly from 2/1 in the resting muscle. The solid frames represent one repeating unit of the structure. The shape of each molecule and the precise relationship between them are not shown. b, The radial projection of two possible models for the cross-bridge arrangement around the thin filament in crab leg muscle in rigor. When a pair of cross-bridges ($L = 1$) occurs symmetrically around the thin filament at intervals of 38 nm, four lattice points (closed circles) out of 28, labelled from 0 to 27 along the genetic helix, are occupied by cross-bridges. This arrangement is the same as the troponin arrangement shown in a ($k = 1$). When two pairs ($L = 2$) of cross-bridges occur at positions adjacent to each other, four more sites (open circles) are occupied. In the latter case, the arrangement can be described by four identical 2/1 helices; each helix includes molecules labelled 0 and 14, 2 and 16, and so on.

helix is deformed slightly from 2/1. This difference is too small to affect the diffraction patterns². We conclude that the troponin arrangement in Ohtsuki's model is valid for the thin filaments of the crab leg muscle, illustrating an application of this method in which intensity modulation is interpreted in terms of helical arrangement of molecules about the thin filament axis.

If we assume that the head portions of myosin molecules are specifically attached to actin subunits, the surface lattice of cross-bridges around thin filaments in rigor (Fig. 2b) is the same as that of troponin molecules with $k = 1$, namely, cross-bridges occur in pairs at intervals of 38 nm. When another pair comes to the next position and both pairs are included at every 38-nm interval (Fig. 2b), the surface lattice can be described with four

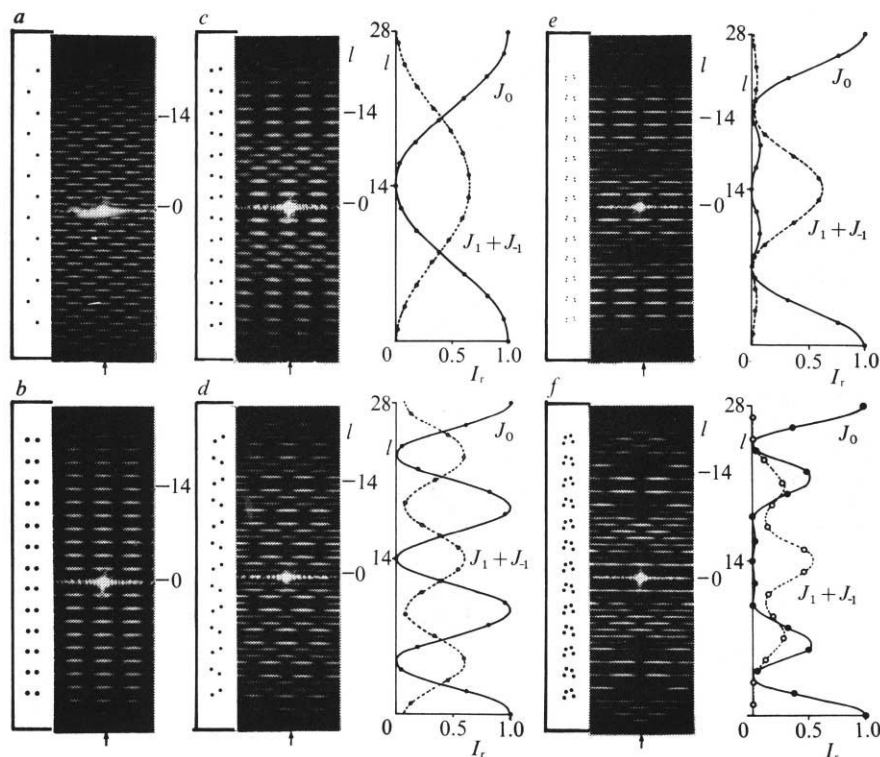


Fig. 3 Models of molecular arrangement around the thin filament and intensity modulations of diffraction patterns predicted from them. Models are *a*, single 2/1 helix; *b*, a pair of subunits in every 38 nm. The arrangement can be described with two identical helices with $\Delta z = 0$ and $\Delta\phi = 0$. *c*, The same as in Fig. 2*a*, *b* (closed circles only). The arrangement can be described with two identical 2/1 helices with $k = 1$. *d*, Two identical 2/1 helices with $k = 3$. *e*, The same as in Fig. 2*b* (closed circles plus open circles). *f*, Offer and Elliott model¹⁰ for the arrangement of cross-bridges in insect flight muscle in rigor. In every 38 nm two pairs are attached to positions next but one to each other. In each set, the left is a model. Closed circles represent troponin and cross-bridges. The right of *a* and *b*, and the middle of *c-f* show optical diffraction patterns obtained from these models. To make higher order layer lines visible, the size of the circles in every model is chosen as that in *e*. The meridian is indicated by an arrow in each pattern. On the right in *c-f*, relative intensity maxima (I_r) of J_0 and $J_1 + J_{-1}$ contributions are plotted against l . These are calculated by equation (1), and the contribution of the form factor is neglected. In reality, unity in intensity in *e* and *f* is stronger by a factor of 4 than that in *c* and *d*. The necessary exposure time for the optical diffraction pattern in *a* is then 4 times as long as those in *b*, *c* and *d*, and 16 times as long as those in *e* and *f*. Note that if two molecules in pairs are axially aligned as in *b*, no odd order reflections can be observed. All the models are based on the actin helix of 28/13. Although the actin helix is slightly different in the crab leg muscle in the resting state, nevertheless, the difference is so small that the diffraction patterns are not affected².

identical 2/1 helices. When the number of pairs is increased to L , modulation terms, for example, for meridional J_0 reflections calculated by equation (1) are given as

$$|a_l^{n=0}|^2 = \sin^2(2L\pi l/28)/\sin^2(\pi l/28) \quad (2)$$

which is an example of Laue function (Fig. 3*e*). Note that the more cross-bridges there are, the smaller the number of reflections on which intensities become concentrated. In the actual pattern from the crab leg muscle in rigor (ref. 1 and Fig. 1*b*), (1) meridional and near-meridional reflections were observed at orders from 2nd to 24th, of 76 nm; (2) meridional reflections at $l = 12, 14$ and 16 are absent, apparently due to a selection rule (if the form factor is diminished there, we cannot explain the fact that near-meridional components at $l = 13$ and 15 are observed in the actual pattern); (3) even order reflections have intensity maxima on the meridian, whereas odd order reflections have not. These features can be explained both by the model with $L = 1$ and by that with $L = 2$. In the diffraction pattern predicted from the $L = 2$ model, a few reflections (especially at $l = 2$) would be almost four times stronger and the rest would be weaker than in the $L = 1$ model. Nevertheless, we cannot decide between the two without measuring intensity distribution. Models with $L \geq 3$ predict extinction of a meridional reflection at $l = 4$ in disagreement with the actual pattern. Any other model can be excluded. As we have pointed out¹, troponin molecules also contribute to these reflections. However, the result is not altered by the troponin contribution in this qualitative analysis of intensity modulation, as troponin does not make an important contribution to these intensities; the ratio of intensity of reflection in resting pattern to the corresponding one in the rigor pattern is smaller than 1/4 and higher order ($l > 9$) reflections are not observed in resting patterns.

To decide between the two possible models, and to determine the spatial relationship between troponin and cross-bridges on the same thin filament and also the size and shape of each molecule, we must make the analysis quantitative by obtaining a cylindrically symmetrical Patterson function⁹.

Offer and Elliott¹⁰ have proposed a model for the cross-bridge arrangement of insect flight muscle in rigor. In this model, two pairs of cross-bridges are included every 38 nm, but the second pair is positioned next but one to the first pair (Fig. 3*f*). The

intensities predicted by the model, again based on intensity modulation calculated by equation (1), seem to be consistent with the recorded X-ray diffraction pattern¹¹. In this muscle, the thin filaments are themselves arranged in a helical manner about the thick filament^{12,13}. The absence of meridional reflections at $l = 2$ and 4 may be explained by the large helix. Lack of reflection at $l = 3$ might support the model, although other possibilities cannot be excluded. The disappearance of several near-meridional components of the diffraction pattern from the muscle after immersion in S-1 solution³ could well be explained by the occurrence of more cross-bridges on the surface lattice with a symmetry of 28/13.

Vibert *et al.*⁴ have recorded a series of reflections from scallop striated muscle in rigor which are thought to be similar to those from crab leg muscle in rigor. They observed an increase in intensities of near-meridional components at 38 nm and at 19 nm, after removal of the regulatory light chains. We might explain these observations by a rearrangement of the cross-bridges: from the arrangement with $L = 1$, for example, to that with $L = 2$. If this hypothesis is true, the intensities of some reflections would be decreased simultaneously.

I thank Professor F. Oosawa and Dr N. Sakabe for helpful comments on earlier drafts of this paper.

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reviews

Nature of chemical elements

G. N. Cantor

The Transcendental Part of Chemistry. By D. Knight. Pp. 289. (Dawson: Folkestone, UK, 1978.) £12.

CHEMISTRY, with all its associations with *praxis*, industry and sombre men in white coats, has or at least has had a 'transcendental' part as fascinating as that of any branch of physics. Yet the standard texts on the history of chemistry have all too often portrayed the development of their subject as an accumulation of experiments which lead directly to improvements in theory. By contrast this revisionist account, like some others of the genre, attempts to show both the subtle interplay between theory and experiment and the influence of metaphysical assumptions on both of these. In so doing Dr Knight and other recent historians of chemistry have uncovered some of the diverse intellectual roots of nineteenth-century chemistry.

This book concentrates on the theories of matter articulated by chemists and, in particular, their views about the nature of chemical elements. Two recurrent positions are identified: while some considered that the profusion of chemical substances were reducible to a very small number of fundamental types of particle—possibly even a single kind—though capable of many different arrangements, others adopted the view that each element was distinct and that it was the chemist's business to ascertain these, their properties and interactions. The relationship of these assumptions to practical chemistry is most clearly seen in the bitter dispute over the value of Prout's hypothesis. As Berzelius and others argued, experimental data refuted the hypothesis and thus seemed strongly to favour the latter position. However, the proponents of the former doctrine did not abandon the field but over a period of time made a strong comeback. The present book ends with Crooke's discovery of primary 'radiant matter' and, from the opposing party, Mendeleev's classification of the elements.

Much of the explicit history of chemistry discussed by Dr Knight is familiar terrain. However, his interpretation of these events takes us far beyond the conventional bounds of chemistry. Matter theory is one of those areas influenced both by scientific and extra-scientific issues, and has thus

provided a particularly interesting topic for historical analysis. For example, chemists of the early nineteenth century were able to draw on a stock of earlier theories such as those of Newton and his elaborators. Moreover, contemporary developments in many branches of science—such as electrochemistry, optics and heat theory—depended on and thereby affirmed certain current theories of matter. Finally, Dr Knight discusses the influences of theological, philosophical and other conceptual issues, citing evidence that some chemists conceived their matter theories as a bulwark against materialism and thus atheism.

The wide scope of this subject is both the book's strength and its weakness. The reader may at times lose the thread of the argument in moving rapidly from one topic to another and from the late

eighteenth to the late nineteenth centuries. In very few cases do the specific relationships between metaphysics and practice emerge with the clarity which these complex issues deserve. Moreover, the philosophical underpinnings of matter theory—such as the definitions of 'matter', 'mechanism' and the role of analogy—deserve sustained analysis.

Despite these shortcomings Dr Knight has written a stimulating and enjoyable book which reflects the awareness among many historians that science has been influenced by extra-scientific factors and that a complex and intriguing relationship exists between metaphysical beliefs, scientific theory and experiment. □

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Solid-state theory

Introduction to Solid State Theory. By O. Madelung. Pp. 486. (Springer: Berlin, Heidelberg and New York, 1978.) DM59; \$29.50.

ONE might argue that solid-state physics is close to the point where the various parts are becoming so disparate and highly developed that there is no place for a relatively short (486 pages) graduate level account. Professor Madelung's book on solid-state theory is intended for graduate students and research workers whose interests are primarily experimental, and provides a coverage of the main current theoretical ideas without using the formalism of modern many-body theory. He has produced a book which is small and coherent enough to be read in conjunction with a graduate course.

The problem of writing a book of this sort is in the danger of either including discussions of a very wide range of subjects all of which are too brief to be useful; or concentrating on some aspects, and so giving an unbalanced account of the subject as a whole. The situation is complicated by the fact that there does not seem to be a consensus of opinion of what every solid-state experimenter should know. There are many obvious candidates such as, pseudo-potential theory, ex-

citons, polarons, BCS theory of superconductivity, Boltzmann transport equation, the Hubbard Model, and so on. But equally there are many more subjects which may or may not deserve more than a passing mention; I would list here the Kondo effect, diffusion in ionic lattices, electron-hole recombination processes at imperfections, Anderson localisation (which Professor Madelung does include) and ferroelectricity and structural phase transitions, spin glasses, charge density waves, the Friedel sum rule and virtual bound states in metallic systems (which he does not). Everyone has his own list!

Pressing on with this argument one should ask: Is it a useful thing to try to do? Do we believe that an experimentalist concerned, say, with light scattering from magnetic insulators ought to know more than is present in the standard undergraduate textbooks on all, or any, of the topics I have mentioned? Also, how far is it useful to separate the theory of the behaviour of an isolated solid from the theory of the solid responding to some probe and if not which experiments should be included?

Professor Madelung believes that there are underlying themes in theoretical solid-state physics, and that this

means that a study of one part of the subject is enriched by a knowledge of the rest. The primary theme that he has chosen is that of elementary excitations with a secondary theme of the dual descriptions of extended and localised states; and this has particular relevance to imperfect systems. The effects of uniform electric and magnetic fields have been included and most of the experiments which are discussed involve either absorption of light or simple transport properties.

The book uses the compact notation of second quantisation but calculations are performed using time-independent perturbation theory. This has the advantage that the new physical ideas

are not obscured by unfamiliar formalism. There is a useful list of problems and topics for discussion to help the student assimilate the material. A further strength of the book is its excellent bibliography which includes a large number of review articles as well as books and original papers.

I find myself convinced that there is a place for a book of this length at this level and would recommend Professor Madelung's book, particularly to new graduate students.

Gillian Gehring

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Psychopharmacology handbook

Handbook of Psychopharmacology. Edited by L. L. Iversen, S. D. Iversen and S. H. Snyder. Vol. 7: Principles of Behavioral Pharmacology. Pp. 453. \$41.40. Vol. 8: Drugs, Neurotransmitters, and Behavior. Pp. 590. \$47.40. Vol. 9: Chemical Pathways in the Brain. Pp. 410. \$35.40. (Plenum: New York and London, 1978.)

WITH these three volumes the Iversen-Snyder *Handbook* moves from the field of basic neuropharmacology into behavioural pharmacology. A total of 30 chapters contributed by 47 authors encompasses a range of subjects from the analysis of operant schedules (Dews and de Weese) to the localisation of gabaminergic neurones (Fonnum and Storm-Mathisen). The contributions are of a high standard and many are highly readable, but it is inevitable in an undertaking of this size that their relationship one to another is often uncertain. Thus, each author attempts to produce a major review of his topic, but is bound to cover material which is also included elsewhere either in these or earlier volumes. Handbooks (and Handbücher) have always suffered from this problem, but of the genre the Iversen-Snyder production has much to commend it. In few other places can one find so much information relevant to the discipline.

Psychopharmacology is nothing if not cross-disciplinary. This should be most evident where two well established disciplines—for example, Psychology and Pharmacology—meet, as they do in volume 7 (Principles of Behavioral Pharmacology). It is interesting to see the extent to which one field has influenced the other. In some cases the impact seems to have been small. In the Dews and De Weese review referred to above drugs appear as

another environmental variable to be accommodated by the same Skinnerian framework that describes other aspects of behaviour. The fact that such agents may be acting upon specific chemical mechanisms within the brain, and that these mechanisms may be regulating specific aspects of behaviour, is regarded as irrelevant to the description of drug action. The purely inductive approach seems, in this case, to act as a defence against asking some obvious, if difficult, questions. From a different psychological viewpoint Milner demonstrates a similar insularity. In giving a useful introductory survey to theories of motivation and learning which would be at home in a textbook of psychology, followed by some neural models, Milner implies that all this may be relevant to recent work on the physiology of central monoamine systems. Yet when it comes to making specific predictions from his own theory, or assessing the progress of other theories of the functions of monoamine neurones, Milner is reticent. It is at this point that the discipline of psychopharmacology (if there is one) gets interesting.

By contrast the work reviewed by Marshall and Teitelbaum (Neuropsychology of Motivated Behaviours) reveals the contribution which a rigorous application of the techniques of neuropsychology to preparations derived from advances in neuropharmacology (6-hydroxy-dopamine induced lesions of dopamine neurones) can make to our understanding of the mechanisms of behaviour. The theoretical implications of this work are considerable, though not developed at length by these authors, and give support to the view that neuroscience is in some respects leaving psychology behind. Other chapters in this volume cover major technical questions (for example, Robbins on motor activity, Mackintosh and others on ethological contributions and methods). Kumar has contributed a succinct but wide-

ranging review of animal models of relevance to psychiatry, attempting to synthesise psychodynamic (for example, object loss), ethological, operant (for example, learned helplessness) and neuropharmacological hypotheses. New dimensions to psychopharmacology are reviewed by Broadhurst (genetics) and Mabry and Campbell (development).

Many issues in volume 7 re-appear in volume 8 (Drugs, Neurotransmitters and Behavior), sometimes in a more controversial form. Here major motivated behaviours (eating, drinking, sleep) or motivational constructs (reward, punishment, fear, frustration) are foci for a discussion of pharmacological, behavioural and often many other aspects. Sometimes the relevance for psychopharmacology is obscure. For example, in a somewhat partisan account of the anatomy of self-stimulation, Routtenberg and Santos-Anderson make a virtue of discounting the neuropharmacological interest of the reward phenomenon. To this reviewer it remains an interesting and viable hypothesis that all reward

Applied group theory

Induced Representations in Crystals and Molecules: Point, Space and Non-rigid Molecule Groups. By S. L. Altmann. Pp. 369. (Academic: London, New York and San Francisco, 1978.) £18; \$32.25. Review by H. C. Longuet-Higgins, *Nature*, **274**, 403 (1978).

DR ALTMANN has drawn the Editor's attention to certain sentences in Professor Longuet-Higgins' review, which he believes are open to criticism.

Professor Longuet-Higgins now writes:

First, my remark that Dr Altmann's groups are "virtually useless for classifying the rotational levels of non-rigid molecules" might have been misconstrued to imply that the book deals explicitly with rotational levels. This it does not do, nor does it claim to do so, although Dr Altmann does give reference to theoretical work which is relevant to the treatment of rotational levels.

Secondly, the statement in the review that the symmetry operations relevant to an overall molecular state must "commute with the full molecular hamiltonian" could be taken to imply that the book defines symmetry operations to commute only with the Born-Oppenheimer hamiltonian; that is not the case.

Finally, my statement that "the book is, unfortunately, riddled with misprints . . . spelling mistakes . . ." could be read as suggesting that such errors were numerous. In fact I found very few.

I apologise unreservedly to Dr Altmann for having allowed these matters to escape my attention.

H. C. Longuet-Higgins

signals are channelled through a final catecholamine element (whether dopaminergic or noradrenergic); whether or not this turns out to be the case it is the association of reward pathways with specific neurohumoural mechanism that must be of particular interest to the psychopharmacologist.

The balance is to some extent redressed by the discussion of Stein *et al.* of the neuropharmacology of reward and punishment. Noradrenaline features prominently, as expected, in relation to the former function, but dopamine now also has a place. The increasingly cogent evidence for a relationship between 5-hydroxytryptamine and punishment mechanisms, and their possible relevance to the actions of the benzodiazepines, is also reviewed. Diverse neuropharmacological approaches to feeding (Hoebel), drinking (Setler) and reproductive behaviour (Meyerson and Eliasson) are comprehensively handled, but many conceptual problems remain. In Jouvet's account of the current state of research on the neurohumoural mechanisms of sleep and waking it is apparent that much that earlier seemed to be clear on the basis of short-term experiments becomes obscure when the ability of the brain in the long term to compensate to apparently decisive insults is revealed.

This volume ends with provocative chapters by Gray on fear and frustration, and Hunter *et al.* on learning and memory. Gray believes that non-reward, punishment and novelty are classes of stimuli that have characteristics in common and may act by a single neurohumoural mechanism. His view that this includes the locus coeruleus projection to septum and hippocampus is in interesting contrast to the views of Stein and others expressed earlier in the volume. Hunter *et al.* review the evidence that noradrenaline and perhaps also 5-hydroxytryptamine and acetylcholine, may be directly involved in the acquisition and storage of new information.

In volume 9 the series returns from the knotty problems of correlating anything with behaviour to the more certain but rapidly expanding field of neurotransmitter pathway mapping. Here the juxtaposition of a number of reviews will be invaluable to the worker who wants a comprehensive account.

The *Handbook of Psychopharmacology* invites comparison with *Psychopharmacology: A Review of Progress 1957-1967* (ed. D. H. Efron, P.H.S. Publication 1836, US Government Printing Office, Washington, 1968) and its successor *Psychopharmacology: A Generation of Progress* (ed. M. A. Lipton, A. D. Di Mascio and K. K. Killam; Raven: New York, 1978). Both the *Handbook* and its competitors are good value. Reviews are presented in more detail, particularly with respect to techniques and their difficulties, in the *Handbook*. Therefore for a shorter review of an aspect with which he is unfamiliar, and particularly with viewpoints and theories, the reader may be advised to go the "Progress" volumes. For a more detailed account the *Handbook* presents much information in a readable form.

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Metal ion solvation

Metal Ions in Solution. By J. Burgess. Pp. 481. (Wiley: New York, London and Toronto, 1978.) £25; \$55.

As implied by the absence of the word "complex" from the title, this book is not concerned with metal complex ions in general. The author has wisely chosen to focus attention on the properties and certain reactions of metal ions that contain one or more solvent molecules as ligands. In view of the abundance of the present literature on inorganic solution chemistry, this is enough for any man to attempt. The result is a book that brings together in convenient form a wide variety of topics which are discussed in sufficient depth to provide a grasp of essentials and a stimulation to further study.

About two-thirds of the book deals

with the study of metal ion solvation by spectroscopic and other techniques, thermodynamic aspects (thermochemistry and redox potentials), hydrolysis and polymerisation. The remaining third is concerned with kinetics and mechanisms—solvent exchange, anation reactions, redox reactions, and reactions of coordinated solvents. The solvolysis and stability constants of complexes are not covered. The treatment of aqueous systems necessarily forms a major part of the discussion, but there are frequent references throughout to non-aqueous solvents and a chapter on mixed solvents and selective solvation. Each topic is introduced at a level that requires a knowledge of basic physical chemistry and then progresses rapidly to the edge of the research literature.

The book is well organised, as shown by the extensive table of contents, and well documented with over 1,700 references and nearly 200 tables of

Vincent J. Maglio & H. B. S. Cooke The Evolution of African Mammals

Current knowledge about the origin and evolution of the Class Mammalia in Africa is summarised in this book. The major geological deposits of the African Cenozoic are reviewed and the broader patterns of faunal evolution are identified. The modern mammalian fauna and environments are discussed, as is man's impact on wildlife. 77 line drawings, 66 halftones, bibliographies. February 1979, £42.00.

Geerat J. Vermeij Biogeography and Adaptation

Patterns of Marine Life

Through this discussion of evolutionary process and adaptive response, Vermeij elucidates the general principles that underlie the great diversity of marine forms found in the world's oceans. 81 halftones, 9 line drawings. February 1979, £17.50.

B. Raymond Fink and Robert J. Demarest Laryngeal Biomechanics

Fink and Demarest offer a new model of laryngeal function and provide both an atlas and a treatise of the behaviour of the larynx, for its size the most complex and versatile mechanical device in the body. *Commonwealth Fund*, 69 halftones, 26 line drawings. January 1979, £21.00.

H. J. Phaff, J. W. Miller & E. M. Mrak The Life of Yeasts

2nd edition, revised & enlarged

The second edition of this book covers recent and major advances in knowledge of the morphology, physiology, genetics and ecology of yeasts, increasingly studied as prototypical eukaryotes. 15 line drawings, 13 halftones. January 1979, £10.50.

HARVARD University Press

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data. The text is set out clearly and seems to be almost entirely free of misprints. There are a few criticisms, of course, but these arise mainly from personal prejudice as to the best way of teaching a particular topic and are perhaps most appropriately aired by direct communication with the author. According to the author the book is intended to occupy the middle ground between the elementary textbook and the erudite review. This is a fair claim. The enterprising undergraduate will find much of use and interest but, con-

sidering all the other parts of chemistry to be studied, will probably find the price beyond his means. Teachers will find the basis for courses at various levels in some important areas where inorganic and physical chemistry overlap. Research workers will find the necessary background (up to the end of 1975) to topics that border on their main fields of interest. There is something for everyone here. **J. S. Coe**

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Aspects of the nude mouse

The Nude Mouse in Experimental and Clinical Research. Edited by J. Fogh and B. C. Giovanella. Pp. 502. (Academic: New York and London, 1978.) \$31; £20.15.

THE most provocative sentence in this interesting book is written by Rygaard. "If the nude mouse had appeared a decade earlier probably nobody would have paid any attention to the odd animal. Had it appeared a decade later most of the problems to which it may offer a solution would have been solved by other means". The last point is surely wrong. The congenitally athymic, nude mouse has been useful in confirming solutions that had been fairly well established using the less perfect T lymphocyte-deficient animals that immunologists have strived so hard to improve but the real value and fascination of this mutant arise from the new problems it has suggested. Thus the appearance of the *nu* gene would have stimulated a flurry of activity had it appeared at any time in the foreseeable future. Immunologists have achieved solutions to some of their problems but the strength of the subject lies in the number of soluble problems on our plate.

This is a timely, useful and well organised collection of chapters on many aspects of the nude mouse. The authors can be cleanly divided into those who regard the nude as a self-replenishing (although hopelessly complicated) test tube for sustaining human tumours and those who exploit the nude to clarify the mechanisms by which normal and nude mice sometimes resist infection, grafts of foreign tissue and tumours. It is clear that the nude mouse reared under conventional conditions is near perfect in its incapacity to reject by an immune response grafts from other strains or from other species as distant as birds and reptiles. However nude mice do not in general show a higher incidence

of either spontaneous or induced tumours. Rygaard and Povlsen found no tumours in a series of nudes observed for 5,600 years of mouse life. According to Stutman's thoughtful analysis the *nu* gene has probably no influence on the incidence of tumours: nudes seem to develop tumours less frequently than other mice because they are usually dead before they are six months old. Of all the observations in disagreement with the doctrine of immune surveillance this is the easiest to grasp. The T cell system of cell-mediated immunity is of established

value in defence against infection and so it is unnecessary to search for additional advantages such as the infanticide of tumours.

If it is not done by T cells do animals use another system for stifling tumours and is this enhanced in the nude mouse? Here and elsewhere a great deal is written about NK cells—apparently lymphocytes possessing Fc receptors which kill a certain range of tumour cells *in vitro*. One is left convinced that the NK cell will come to be regarded as either the major immunological discovery of this decade or as a remarkable fiasco.

The ingenuity applied to the exploitation of the nude mouse is only just beginning. Two separate strains of nude, athymic rats have recently been discovered. These may or may not share precisely the same genetic defect with each other and with the nude mouse. In their turn they will confirm much of the new information gleaned from the nude mouse and will suggest fresh problems of their own.

W. L. Ford

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Catalytic processes

Chemistry of Catalytic Processes. By B. C. Gates, J. R. Katzer and G. C. A. Schuit. Pp. 464. (McGraw-Hill: New York, London and Toronto, 1978.) £21.35.

THIS is an interesting book which brings together the chemistry of catalysis and many of the concepts and techniques of importance to the operation of processes on the industrial scale. The plan adopted is to cover just five of the most significant types of catalytic processes and use them as a framework upon which to build. This approach might give the impression that the treatment of the subject would be more specialised and less general than is appropriate for a book of this kind, but, in fact, such an impression would be completely false. A great deal of fundamental chemistry is introduced as a basis for discussion of the chosen processes.

The chapter on cracking provides an account of catalysis on amorphous and crystalline acidic surfaces, describes the chemistry associated with carbonium ion intermediates and in addition it covers aspects of reactor design needed for catalyst regeneration to avoid loss of activity from coking. The second chapter is concerned with a

number of reactions on transition metal complexes falling mainly under the classification of homogeneous catalysis; the basic chemistry included in this chapter deals with structure, bonding and reactions of organometallic complexes.

There is a very substantial amount of relevant fundamental material in the chapter on reforming. The treatment ranges from discussions of bonding in metals and alloys to consideration of the structures of typical oxide supports and from evidence for the nature of hydrogen adsorption on clean surfaces to the mechanisms of various hydrocarbon reactions on metals and on alumina.

The final chapters cover oxidation as exemplified by the ammoxidation of propylene and hydrodesulfurisation. Once again the same general combination of fundamental chemistry with relevant process engineering is provided in these chapters.

Taken as a whole this is a stimulating book which should appeal to a wide range of chemists and engineers who have a concern with reactions on surfaces. The novel approach and the skilful blend of recent ideas and developments with the more traditional aspects of the subject make the volume both attractive and timely.

Charles Kembell

Charles Kembell is Professor of Chemistry at the University of Edinburgh, Scotland.

announcements

Awards

The Royal Society has recently awarded the following medals: The Copley Medal to **Professor R. B. Woodward**; Royal Medals to **Professors R. A. Gregory, A. Salaam and T. Kilburn**; The Davy Medal to **Professor A. Eschenmoser**; The Darwin Medal to **Dr G. Pontecorvo**; The Leverhulme Medal to **Sir Frederick Warner**; The Mullard Medal to **Dr J. W. Black**; The Esso Medal to **A. G. Frame, J. M. Laithwaite, A. D. Evans, Dr J. F. W. Bishop and Dr T. N. Marsham**; The Rumford Medal to **Sir George Porter**; The Hughes Medal to **Professor W. Cochran**.

The following awards of the New York Academy of Science were made recently: The Distinguished Service Award of The New York Academy of Sciences was presented posthumously to **Margaret Mead**, eminent anthropologist and humanist, in recognition of her outstanding contributions to science and society; **Harrison Brown** has received The New York Academy of Sciences Award; **Anna Goldfeder** has received the Academy's Presidential Award; **Philip Leder** has received The New York Academy of Sciences Award in Biological and Medical Sciences; **Michael E. Fisher** has received The New York Academy of Sciences Award in Physical and Mathematical Sciences; **Michael Cole** has received The New York Academy of Sciences Award in Behavioral Sciences; **Peter Rentzepis** has received the A. Cressy Morrison Award in the Natural Sciences; **John J. McKetta** has received The Boris Pregel Award for Applied Science and Technology; **Paul Edward Garber** has received the I. B. Laskowitz Award for Research in Aerospace Engineering Sciences, Support Systems and Components; **Barbara McClintock** has received the Louis and Bert Freedman Foundation Award for Research in Biochemistry; **Victor Sidel** has received the Sarah L. Poiley Memorial Award; **Patrick Eggena** has received the Dr Harold Lamport Award; **Orville L. Chapman** has received the Gregory and Freda Halpern Award in Photochemistry.

Professor J. H. Burn, Emeritus Professor of Pharmacology, Oxford University, has been awarded the first Wellcome Gold Medal of the British Pharmacological Society.

The 1979 Israel Prize in the Life Sciences has been awarded to **Professor Hans Lindner**, Head of the Weizmann Institute's Hormone Research Department, for his outstanding achievements and most important contributions to endocrinology and the biology of reproduction.

The American Geophysical Union (AGU) has presented **Hsieh W. Shen**, Professor of Civil Engineering, Colorado State University, with the Robert E. Horton Award.

The first Wellcome Trust Award for Research in Biochemistry Related to Medicine has been made to **Dr D. J. H. Brock**, Reader in Human Genetics in the University of Edinburgh.

R. W. Whorlow, Senior Lecturer in the University of Surrey's Department of Physics, has received the first Annual Award of the British Society of Rheology, in recognition of his outstanding services to the Society for his work in the teaching and science of rheology.

Appointments

The President of the Royal Society, the Rt. Hon. the Lord Todd, OM, has appointed the following Vice-Presidents for the year ending 30 November 1979: **Dr B. J. Mason, CB**, Treasurer of the Royal Society; director-general of the Meteorological Office; **Professor D. C. Phillips**, Biological Secretary of the Royal Society; professor of molecular biophysics at the University of Oxford; **Dr T. M. Sugden, CBE**, Physical Secretary of the Royal Society; master of Trinity Hall, Cambridge; **Dr M. G. P. Stoker, CBE**, Foreign Secretary of the Royal Society; director of the Imperial Cancer Research Fund Laboratories; **Professor P. Allen**, professor and head of the department of geology and director of the sedimentology research laboratory in the University of Reading; **Sir Arnold Hall**, chairman and managing director, Hawker Siddeley Group Limited; **Professor D. C. Smith**, Melville Wills professor of botany and director of biological sciences in the University of Bristol; **Sir Michael Woodruff**, emeritus professor of surgery in the University of Edinburgh.

Professor Dennis J. Greenland has been appointed Deputy Director-General of the International Rice Research Institute in the Philippines.

Tony Benn, Secretary of State for Energy, has appointed **W. P. Blair** to be a member of the Advisory Council on Energy Conservation. Mr Blair is an Executive Council member of the Electrical, Electronic, Telecommunications and Plumbing Union (EETPU).

Dr Page Faulk, formerly Director of the Division of Developmental Immunology to The Medical University of South Carolina, US, has been appointed as Director of The Blond McIndoe Centre with effect from the 1st January 1979 and the Royal College of Surgeons of England have conferred on him the title of Honorary Research Professor.

Dr Charles M. Brown, senior lecturer in Biological Sciences at the University of Dundee, has been appointed to a new Chair in the Department of Brewing and Biological Sciences at Heriot-Watt University, Edinburgh. He will take up his appointment on 1 July 1979.

On 1 July 1979, **Dr Daniel A. Pollen** will become Director of Neurological Research and Chairman of the Division of Neurobiology at the Barrow Neurological Institute of St Joseph's Hospital and Medical Center in Phoenix, Arizona. Dr Pollen leaves the Neurosurgical Service of the Massachusetts General Hospital and Harvard Medical School where he was Associate Professor of Physiology in the Department of Surgery.

Dr J. W. Bridges, Reader in the University of Surrey's Department of Biochemistry and Director of the recently founded Institute of Industrial and Environmental Health and Safety, has been appointed to the new Chair in Toxicology—the only University Chair in Toxicology in the UK.

Joel L. Lebowitz, Professor of Mathematics and Physics and Director of the Center for Mathematical Sciences Research at Rutgers University, has been installed as president at The New York Academy of Sciences' 161st Annual Meeting held at The American Museum of Natural History on 5 December 1979.

Edward R. Hitchcock, a Birmingham medical graduate of 1952, has returned to the University as Professor and Head of the Department of Neurosurgery.

The Trustees of the Nuffield Foundation have approved a scheme devised by the Foundation's Committee on Rheumatism Research for the appointment of Senior Visiting Fellows in Rheumatism Research. The Committee hopes that the first appointment under this scheme will be made in time for the beginning of the academic year 1979-80. These Fellows will normally be based at a suitable laboratory in the UK during the tenure of their fellowship, but will be expected to visit most other centres of rheumatism research in Britain during the period of their appointment. Correspondence should be addressed to John Maddox, Director of the Nuffield Foundation, Nuffield Lodge, Regents Park, London NW1.

Meetings

2-6 April, **Appropriate Energy Technology for Development**, Glasgow (Dr J. Twidell, Appropriate Technology Seminar, Dept. of Applied Physics, University of Strathclyde, Glasgow, UK).

3-5 April, **Analytical Symposia**, London (T. Goodall, Pye Unicam Ltd, York Street, Cambridge, UK).

4-6 April, **Cell Adhesion and Motility**, Glasgow (Dr J. D. Pitts, Dept of Biochemistry, University of Glasgow, UK).
18-20 April, **ELSE, 4th General Assembly**, Budapest (Miss N. Morris, 30 Longdown Road, Lower Bourne, Farnham, Surrey, UK).

19-20 April, **Superconducting Electrical Machines**, Oxford (Meetings Officer, The Institute of Physics, 47 Belgrave Square, London SW1, UK).

15 May, **Cancer—The Patient**, London (The Secretary, The Marie Curie Memorial Foundation, 124 Sloane Street, London SW1, UK).

16 May, **Industrial Applications of Particle Beams for Materials Analysis**, London (Meetings Officer, The Institute of Physics, 47 Belgrave Square, London SW1, UK).

16-17 May, **32 Chemists' Conference**, Scarborough (K. Speight, British Steel Corporation, Sheffield Laboratories, Hoyle Street, Sheffield, UK).

2-13 July, **Factors Influencing Urban Design: A Systems Approach**, Louvain-la-Neuve (Dr P. Laconte, Expansion Dept., Université Catholique de Louvain, 13 Ave G. Lemaitre, B-1348 Louvain-la-Neuve, Belgium).

4-6 July, **194th Meeting of the Society for Experimental Biology**, Belfast (Dr M. W. Steer, Dept of Botany, Queen's University, Belfast, UK).

11-13 July, **Polypeptides Hormones**, Baltimore (E. G. Bassett, Miles Laboratories Inc., PO Box 40, Elkhart, Indiana, 46515).

15-22 July, **Bioethics and Public Policy**, Boulder (A. Perry, Institute of Society, Ethics and the Life Sciences, 360 Broadway, Hastings-on-Hudson, New York 10706).

16-19 July, **Endocrinology '79**, London (Conference Secretariat, The Endocrine Unit, Royal Postgraduate Medical School, Du Cane Road, London, W1, UK).

17-20 July, **INTERMAG—Magnetism and Magnetic Materials**, New York (Dr E. F. Luborsky, General Electric R&D Center, PO Box 8, Schenectady 12301).

19-21 July, **Genetic Aspects of Fertility and Fetal Wastage**, Southampton (European Society of Human Genetics, Dr M. Seabright, Wessex Regional Cytogenetics Unit, Salisbury General Hospital, Salisbury, UK).

1-3 August, **Theoretical and Practical Aspects of Recombinant DNA Techniques**, Sao Paulo (Prof. F. J. S. Lara, Dept. of Biochemistry, Institute of Chemistry, University of Sao Paulo and Institute Butantan, Sao Paulo 20780, Brazil).

13-24 August, **The Scientific and Mathematical Foundations of Engineering Acoustics**, Massachusetts (R. H. Lyon, Massachusetts Institute of Technology, Room 3-366, Dept. of Mechanical Engineering, Cambridge, Massachusetts 02139).

15-17 August, **20th Canadian High Polymer Forum**, Quebec (Dr I. H. McEwan, Canadian Industries Ltd, Paint Research Laboratory, 1330 Castlefield Avenue, Toronto, Ontario, Canada M6B 4B3).

26-31 August, **Biochemical Development of Nervous Tissue in Culture**, Tel Aviv (Dr E. Giacobini, Dept. of Biobehavioral Sciences, University of Connecticut, Storrs, Connecticut).

27-31 August, **The Teaching of Chemistry—Interaction between Secondary and Tertiary Levels**, Dublin (Professor D. J. Waddington, University of York, UK).

28 August-1 September, **Neurochemistry of the Retina**, Athens (Dr N. G. Bazan, Instituto de Investigaciones Bioquímicas, Univ. Nacional del Sur—Conicet, Av. Alem 1253, 800—Bahia Blanca, Argentina).

Reports & Publications

UK & Ireland—January

- Hebridean Naturalist*, Vol. 1, No. 1/2, Summer/Winter 1978. Edited by I. Stewart Angus. Pp. 1-84. (Stornaway, Isle of Lewis: Western Isles Natural History Society, 5 Rathad Na Muilne, 1978.) £2.50. [151]
National Institute for Medical Research. Report for 1977-78. Pp. iii+133. (London: Medical Research Council, 1978.) [161]
Health and Safety Executive. Highly Flammable Materials on Construction Sites. Pp. iv+46. (London: HMSO, 1978.) £1 net. [171]
National Gallery Technical Bulletin, Vol. 2, 1978. Pp. 76. (London: Publications Department, The National Gallery, 1978.) £2. [171]
Department of the Environment. A Report on Captive Hawks. Pp. 10. (London: Department of the Environment, 2 Marsham Street, SW1, 1979.) [171]
Nuclear Power: Its Integration Into a Large Utility. By Glyn England. (Based on an Address to the British

Nuclear Energy Society at the Institution of Civil Engineers, London, on 25 May 1978.) Pp. 28. (London: Press and Publicity Office, Central Electricity Generating Board, 15 Newgate Street, EC1, 1978.) [181]

Health Service Commissioner. First Report for Session 1978-79. Investigations completed—August to November 1978. Pp. 254. (London: HMSO, 1978.) £3.50 net. [191]

Innovative Technologies for a Future Society. By Professor Carl-Göran Heden. Pp. 24. (Scott Bader Commonwealth Monograph No. 9.) (Wellingborough, Northants: The Scott Bader Commonwealth, Ltd., Wollaston, 1979.) [221]

A Key to the British Species of Freshwater Triclad (Turbellaria, Paludicola). By T. B. Reynoldson. (Scientific Publication No. 23.) Pp. 31. (Far Sawrey, Ambleside, Cumbria: Freshwater Biological Association, The Ferry House, 1978.) £1.30 [221]

Universities Research Reactor. Annual Report, 1977-1978. Pp. iii+38. (Risley, Warrington: Universities Research Reactor, 1979.) [221]

Proceedings of the Royal Irish Academy. Section A: Mathematical and Physical Sciences. Vol. 78. No. 17: On the Use of the Renormalisation Group Near the Electron's Mass Shell. By C. Nash and R. L. Stuller. Pp. 149-156. £1.10. No. 18: The Use of Meixner Functions in the Identification of Heavily-Damped Systems. By J. C. L. Dooge and B. J. Garvey. Pp. 157-179. £2.40. (Dublin: Royal Irish Academy, 1978.) [221]

Biotechnology Letters, Vol. 1, Part 1. Pp. 1-59. Published monthly. Annual subscription: Individuals £15 (\$30); Libraries £50 (\$100). (Kew, Surrey: Science and Technology Letters, 12 Clarence Road, 1979.) [221]

Health and Safety Executive. Health and Safety: Manufacturing and Service Industries 1977. A Report of the Work of HM Factory Inspectorate incorporating the Annual Report of HM Inspector of Explosives. Pp. vii+84. (London: HMSO, 1978.) £2.75 plus postage. [231]

Guide to Government Department and Other Libraries and Information Bureaux. Pp. 92. (London: The British Library, Science Reference Library, 1978.) £3. [251]

Department of Health and Social Security. Research and Development Report and Handbook 1978. (A Report and List of Projects Supported by the Department in the financial year 1977-78. Pp. v+167. (London: HMSO, 1978.) £4 net. [261]

Motorways and Agriculture: Severance. By Michael A. B. Boddington, Alan S. Hearne and P. M. K. Leat. (Rural Planning Services, Ltd. Publication No. 5.) Pp. vi+33. (Homer, Ipsden, Oxon: Rural Planning Services, Ltd., 1978.) £2 net. [261]

Bulk Refractories Industry: Energy Conservation and Utilisation in the Bulk Refractories Industry. (Energy Audit Series, No. 4.) Pp. 62. (London: Library, Department of Energy, Thames House South, Millbank, 1978.) gratis. [261]

University College London. Annual Report, 1977-1978. Pp. 137. (London: University College London, 1979.) [291]

International Vegetarian Health Food Handbook 1979-80. Pp. 208. (Altrincham, Cheshire: The Vegetarian Society, Parkdale, Dunham Road, 1979.) £1.20 + 15p postage. [301]

Philosophical Transactions of the Royal Society of London. A: Mathematical and Physical Sciences. Vol. 290. No. 1373: A Precision Determination of the Lamb Shift in Hydrogen. By G. Newton, D. A. Andrews, and P. J. Unsworth. Pp. 373-404. UK £2.10; Overseas £2.20. Vol. 290. No. 1374: Resonance Tunnelling of Waves in a Stratified Cold Plasma. By K. G. Budden, FRS. Pp. 405-433. UK £1.90; Overseas £2. Vol. 290. No. 1375: Discrete Symmetric Dynamical Systems at the Main Resonances with Applications to Axisymmetric Galaxies. By F. Verhulst. Pp. 435-465. UK £2.05; Overseas £2.15. (London: The Royal Society, 1979.) [311]

Other countries—January

Australia: Commonwealth Scientific and Industrial Research Organization. Annual Report of the Division of Textile Physics, 1977/1978. Pp. 53. (Ryde: CSIRO, 338 Blaxland Road, 1978.) [81]

Employment and Environment. Pp. 68. (Paris: OECD, 2, rue André Pascal, 1978.) F18; £2.20; \$4.50. [81]

Bulletin of the American Museum of Natural History. Vol. 161, Article 4: Revision of the *Myiarchus* Flycatchers of South America. By Wesley E. Lanyon. Pp. 427-628. (New York: American Museum of Natural History, 1978.) \$12.70. [81]

A Report to the President and Congress on the Status of Health Professions Personnel in the United States. (Hyattsville, Md.: US Department of Health, Education and Welfare, Public Health Service, Health Resources Administration, 3700 East-West Highway, 1978.) [9]

US Department of the Interior: Geological Survey. Professional Paper 1064-A: Mixed-Layer Clay in the Pierre Shale and Equivalent Rocks, Northern Great Plains Region. By Leonard G. Schultz. Pp. iv+28. (Washington, DC.: US Government Printing Office, 1978.) [91]

World Health Organization. International Classification of Diseases Ninth Revision: Basic Tabulation List with Alphabetical Index. Pp. 331. Geneva: WHO London: HMSO, 1978.) Sw.fr. 15. [111]

Académie Royale de Belgique. Mémoires de la Classe des Lettres. T. LXIII, Fasc. 3: Étude sur la Confédération Bédouienne (447/6-386)—Son Organisation et son Administration. Par Pierre Salmon. Pp. 269. (Bruxelles: Académie Royale de Belgique, 1978.) [121]

Energy, Mines and Resources Canada. Geological Survey of Canada. Bulletin 284: Lower Ordovician Acratarchs and Trilobites from Bell Island, Eastern Newfoundland. By W. T. Dean and F. J. Martin. Pp. 35 (7 plates). (Ottawa: Geological Survey of Canada, 1978.) \$3.50. [151]